TABLE OF CONTENTS

UNITED STATES PATENT APPLICATION

OF

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FOR

β-hCG PEPTIDES, FRACTIONS OF hCG SOURCES AND THERAPEUTIC
METHODS EMPLOYING SUCH PEPTIDES AND/OR FRACTIONS

INTELLECTUAL PROPERTY / TECHNOLOGY LAW

β-hCG PEPTIDES, FRACTIONS OF hCG SOURCES AND THERAPEUTIC METHODS EMPLOYING SUCH PEPTIDES AND/OR FRACTIONS

This application is a continuation-in-part of International Patent Applications PCT/US97/11210, entitled "Treatment and Prevention of Cancer by
5   Administration of Derivatives of Human Chorionic Gonadotropin", PCT/US97/11209, entitled "Methods of Promoting Hematopoiesis using Derivatives of Human Chorionic Gonadotropin", PCT/US97/11448 "Treatment and Prevention of Wasting Syndrome Based on Administration of Derivatives of Human Chorionic Gonadotropin", and PCT/US97/11202, entitled "Treatment and
10   Presentation of HIV Infection by Administration of Derivatives of Human Chronic Gonadotropin," all filed on 24 June 1998, and all of which are incorporated herein by reference in their entirety.

The following applications are also incorporated herein by reference: U.S. Patent Applications entitled "Treatment and Prevention of Cancer by
15   Administration of Derivatives of Human Chorionic Gonadotropin", Serial No. 08/709,925, filed September 9, 1996; "Methods of Promoting Hematopoiesis Using Derivatives of Human Chorionic Gonadotropin", by Gallo et al., Serial No. 08/709,924, filed September 9, 1996; "Treatment and Prevention of Wasting Syndrome Based on Administration of Derivatives of Human Chorionic
20   Gonadotropin" by Gallo et al., Serial No. 08/709,933, filed September 9, 1996; and "Treatment and Prevention of HIV Infection by Administration of Derivatives of Human Chorionic Gonadotropin", Serial No. 08/709,984, filed September 9, 1996; and U.S. Patent Applications entitled "Treatment and Prevention of Cancer by Administration of Derivatives of Human Chorionic
25   Gonadotropin", by Gallo et al., Serial No. 08/669,676, filed June 24, 1996; "Methods of Promoting Hematopoiesis Using Derivatives of Human Chorionic Gonadotropin", by Gallo et al., Serial No. 08/669,654, filed June 24, 1996; "Treatment and Prevention of Wasting Syndrome Based on Administration of Derivatives of Human Chorionic Gonadotropin" by Gallo et al., Serial No.

1

08/669,675, filed June 24, 1996; and "Treatment and Prevention of HIV Infection by Administration of Derivatives of Human Chorionic Gonadotropin" by Gallo et al., Serial No. 08/669,681, filed June 24, 1996.

1 FIELD OF THE INVENTION

5 The present invention relates to peptides of one or more portions of the human chorionic gonadotropin β-chain as well as methods for treatment and prevention of diseases, including HIV infection, cancer and wasting syndrome, and methods of promoting hematopoiesis using human chorionic gonadotropin, employing the β-chain of human chorionic gonadotropin, peptides containing a sequence of 10 one or more portions of the β-chain of human chorionic gonadotropin and derivatives and analogues thereof. The invention further relates to fractions of sources and or preparations of human chorionic gonadotropin, such as fractions of human early pregnancy urine, which fractions have anti-HIV activity, anti-cancer activity, anti-wasting activity, and/or pro-hematopoietic activity. The 15 present invention further relates to pharmaceutical compositions for treating and/or preventing HIV infection, cancer, and/or wasting, and/or for promoting hematopoiesis.

2 BACKGROUND OF THE INVENTION

2.1 THE HUMAN IMMUNODEFICIENCY VIRUS

20 The human immunodeficiency virus (HIV) has been implicated as the primary cause of the slowly degenerative immune system disease termed acquired immune deficiency syndrome (AIDS) (Barre-Sinoussi, F., et al., 1983, *Science* 220:868-870; Gallo, R., et al., 1984, *Science* 224:500-503). There are at least two distinct types of HIV: HIV-1 (Barre-Sinoussi, F., et al., 1983, *Science* 25 220:868-870; Gallo, R., et al., 1984, *Science* 224:500-503) and HIV-2 (Clavel, F., et al., 1986, *Science* 233:343-346; Guyader, M., et al., 1987, *Nature* 326:662-669). Further, a large amount of genetic heterogeneity exists within

2

populations of each of these types. In humans, HIV replication occurs prominently in CD4$^+$ T lymphocyte populations, and HIV infection leads to depletion of this cell type and eventually to immune incompetence, opportunistic infections, neurological dysfunctions, neoplastic growth, and

5 ultimately death.

HIV is a member of the lentivirus family of retroviruses (Teich, N., et al., 1984, *RNA Tumor Viruses*, Weiss, R., et al., eds., CSH-Press, pp. 949-956). Retroviruses are small enveloped viruses that contain a single-stranded RNA genome and replicate via a DNA intermediate produced by a virally-encoded

10 reverse transcriptase, an RNA-dependent DNA polymerase (Varmus, H., 1988, *Science* 240:1427-1439).

The HIV viral particle comprises a viral core, composed in part of capsid proteins, together with the viral RNA genome and those enzymes required for early replicative events. Myristylated gag protein forms an outer shell around

15 the viral core, which is, in turn, surrounded by a lipid membrane envelope derived from the infected cell membrane. The HIV envelope surface glycoproteins are synthesized as a single 160 kilodalton precursor protein which is cleaved by a cellular protease during viral budding into two glycoproteins, gp41 and gp120. gp41 is a transmembrane glycoprotein and gp120 is an

20 extracellular glycoprotein which remains non-covalently associated with gp41, possibly in a trimeric or multimeric form (Hammarskjold, M., & Rekosh, D., 1989, *Biochem. Biophys. Acta* 989:269-280).

HIV is targeted to CD4$^+$ cells because a CD4 cell surface protein (CD4) acts as the cellular receptor for the HIV-1 virus (Dalgleish, A., et al., 1984, *Nature*

25 312:763-767; Klatzmann et al., 1984, *Nature* 312:767-768; Maddon et al., 1986, *Cell* 47:333-348). Viral entry into cells is dependent upon gp120 binding the cellular CD4 receptor molecules (McDougal, J.S., et al., 1986, *Science* 231:382-385; Maddon, P.J., et al., 1986, *Cell* 47:333-348), explaining HIV's tropism for

3

CD4$^+$ cells, while gp41 anchors the envelope glycoprotein complex in the viral membrane. While these virus:cell interactions are necessary for infection, there is evidence that additional virus:cell interactions are also required.

2.2   HIV TREATMENT

5   HIV infection is pandemic and HIV-associated diseases represent a major world health problem. Although considerable effort is being put into the design of effective Therapeutics, currently no curative anti-retroviral drugs against AIDS exist. In attempts to develop such drugs, several stages of the HIV life cycle have been considered as targets for therapeutic intervention (Mitsuya, H., et al.,

10   1991, *FASEB J.* 5:2369-2381). Many viral targets for intervention with HIV life cycle have been suggested, as the prevailing view is that interference with a host cell protein would have deleterious side effects. For example, virally encoded reverse transcriptase has been one focus of drug development. A number of reverse-transcriptase-targeted drugs, including 2',3'-

15   dideoxynucleoside analogs such as AZT, ddl, ddC, and d4T have been developed which have been shown to been active against HIV (Mitsuya, H., et al., 1991, *Science* 249:1533-1544).

The new treatment regimens for HIV-1 show that a combination of anti-HIV compounds, which target reverse transcriptase (RT), such as azidothymidine

20   (AZT), lamivudine (3TC), dideoxyinosine (ddl), dideoxycytidine (ddC) used in combination with an HIV-1 protease inhibitor have a far greater effect (2 to 3 logs reduction) on viral load compared to AZT alone (about 1 log reduction). For example, impressive results have recently been obtained with a combination of AZT, ddl, 3TC and ritonavir (Perelson, A.S., et al., 1996,

25   *Science* 15:1582-1586). However, it is likely that long-term use of combinations of these chemicals will lead to toxicity, especially to the bone marrow. Long-term cytotoxic therapy may also lead to suppression of CD8$^+$ T cells, which are essential to the control of HIV, via killer cell activity (Blazevic,

4

V., et al., 1995, *AIDS Res. Hum. Retroviruses* 11:1335-1342) and by the release of suppressive factors, notably the chemokines Rantes, MIP-1α and MIP-1β (Cocchi, F., et al., 1995, *Science* 270:1811-1815). Another major concern in long-term chemical anti-retroviral therapy is the development of HIV mutations

5 with partial or complete resistance (Lange, J.M., 1995, *AIDS Res. Hum. Retroviruses* 10:S77-82). It is thought that such mutations may be an inevitable consequence of anti-viral therapy. The pattern of disappearance of wild-type virus and appearance of mutant virus due to treatment, combined with coincidental decline in CD4$^+$ T cell numbers strongly suggests that, at least with

10 some compounds, the appearance of viral mutants is a major underlying factor in the failure of AIDS therapy.

Attempts are also being made to develop drugs which can inhibit viral entry into the cell, the earliest stage of HIV infection. Here, the focus has thus far been on CD4, the cell surface receptor for HIV. Recombinant soluble CD4, for

15 example, has been shown to inhibit infection of CD4$^+$ T cells by some HIV-1 strains (Smith, D.H., et al., 1987, *Science* 238:1704-1707). Certain primary HIV-1 isolates, however, are relatively less sensitive to inhibition by recombinant CD4 (Daar, E., et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:6574-6579). In addition, recombinant soluble CD4 clinical trials have produced

20 inconclusive results (Schooley, R., et al., 1990, *Ann. Int. Med.* 112:247-253; Kahn, J.O., et al., 1990, *Ann. Int. Med.* 112:254-261; Yarchoan, R., et al., 1989, Proc. Vth Int. Conf. on AIDS, p. 564, MCP 137).

The late stages of HIV replication, which involve crucial virus-specific processing of certain viral encoded proteins, have also been suggested as

25 possible anti-HIV drug targets. Late stage processing is dependent on the activity of a viral protease, and drugs are being developed which inhibit this protease (Erickson, J., 1990, *Science* 249:527-533).

Recently, chemokines produced by CD8^{+} T cells have been implicated in suppression of HIV infection (Paul, W.E., 1994, *Cell* 82:177; Bolognesi, D.P., 1993, *Semin. Immunol.* 5:203). The chemokines RANTES, MIP-1α and MIP-1β, which are secreted by CD8^{+} T cells, were shown to suppress HIV-1 p24 antigen

5 production in cells infected with HIV-1 or HIV-2 isolates *in vitro* (Cocchi, F, et al., 1995, *Science* 270:1811-1815). Thus, these and other chemokines may prove useful in therapies for HIV infection. The clinical outcome, however, of all these and other candidate drugs is still in question.

Attention is also being given to the development of vaccines for the treatment

10 of HIV infection. The HIV-1 envelope proteins (gp160, gp120, gp41) have been shown to be the major antigens for anti-HIV antibodies present in AIDS patients (Barin et al., 1985, *Science* 228:1094-1096). Thus far, therefore, these proteins appear to be the most promising candidates to act as antigens for anti-HIV vaccine development. Several groups have begun to use various portions of

15 gp160, gp120, and/or gp41 as immunogenic targets for the host immune system. See for example, Ivanoff, L., et al., U.S. Pat. No. 5,141,867; Saith, G., et al., WO92/22,654; Shafferman, A., WO91/09,872; Formoso, C., et al., WO90/07,119. Vaccines directed against HIV proteins are problematic in that the virus mutates rapidly rendering many of these vaccines ineffective. Thus,

20 although a great deal of effort is being directed to the design and testing of anti-retroviral drugs, effective, non-toxic treatments are still needed.

2.3   HEMATOPOIETIC CELL PRODUCTION

The morphologically recognizable and functionally capable cells circulating in blood include erythrocytes, neutrophilic, eosinophilic, and basophilic

25 granulocytes, B-, T-, non B-, non T-lymphocytes, and platelets. These mature hematopoietic cells derive from and are replaced, on demand, by morphologi-cally recognizable dividing precursor cells for the respective lineages such as erythroblasts for the erythrocyte series, myeloblasts, promyelocytes and

myelocytes for the granulocyte series, and megakaryocytes for the platelets. The precursor cells derive from more primitive cells that can simplistically be divided into two major subgroups: stem cells and progenitor cells (for review, see Broxmeyer, H.E., 1983, "Colony Assays of Hematopoietic Progenitor Cells

5 and Correlations to Clinical Situations," *CRC Critical Reviews in Oncology/Hematology* 1:227-257).

The definitions of stem and progenitor cells are operational and depend on functional, rather than on morphological, criteria. Stem cells have extensive self-renewal or self-maintenance capacity (Lajtha, L.G., 1979, *Differentiation*

10 14:23), a necessity since absence or depletion of these cells could result in the complete depletion of one or more cell lineages, events that would lead within a short time to disease and death. Some of the stem cells differentiate upon need, but some stem cells or their daughter cells produce other stem cells to maintain the pool of these cells. Thus, in addition to maintaining their own

15 kind, pluripotential stem cells are capable of differentiation into several sub-lines of progenitor cells with more limited self-renewal capacity or no self-renewal capacity. These progenitor cells ultimately give rise to the morphologically recognizable precursor cells. The progenitor cells are capable of proliferating and differentiating along one, or more than one, of the myeloid

20 differentiation pathways (Lajtha, L.G. (Rapporteur), 1979, *Blood Cells* 5:447).

A variety of infectious agents, genetic abnormalities and environmental factors can cause a deficiency in one or more hematopoietic cell types. For example, hematological abnormalities have been observed in HIV-1 infected individuals (the human immunodeficiency virus (HIV) has been implicated as the primary

25 cause of the slowly degenerative immune system disease termed acquired immune deficiency syndrome (AIDS) (Barre-Sinoussi, F., et al., 1983, *Science* 220:868-870; Gallo, R., et al., 1984, *Science* 224:500-503)) , particularly in the late stages of disease (Lunardi-Iskandar, Y. et al., 1989, *J. Clin. Invest* 83:610-615). These abnormalities include a reduction in $CD4^+$ T cells as well as

cytopenias of one or more hematopoietic lineages, often associated with bone marrow morphologic abnormalities and deficient progenitor cell growth (Lunardi-Iskandar, Y. et al., 1989, *J. Clin. Invest* 83:610-615; Louache, F. et al., 1992, *Blood* 180:2991-2999). Idiopathic thrombocytopenic purpura (ITP),

5   characterized by significant reduction in platelet numbers, often afflicts subjects infected with HIV (Ballem, P.J. et al., 1992, *N. Engl. J. Med.* 327:1779). The destruction of platelets in sufferers of ITP appears to be mediated by platelet associated autoantibodies (Berchtold, P. and Wenger, M., 1993, *Blood* 81:1246; Ballem, P.J. et al., 1987, *J. Clin. Invest.* 80:33). Thus, because management of

10   ITP generally involves immunosuppression, treatment of ITP in HIV infected patients is complicated as administration of immunosuppressive drugs is extremely detrimental in HIV infection.

Additionally, chemotherapy and radiation therapy used in the treatment of cancer and certain immunological disorders can cause pancytopenias or

15   combinations of anemia, neutropenia and thrombocytopenia. Thus, the increase or replacement of hematopoietic cells is often crucial to the success of such treatments. (For a general discussion of hematological disorders and their causes, *see, e.g.*, "Hematology" *in* Scientific American Medicine, E. Rubenstein and D. Federman, eds., Volume 2, chapter 5, Scientific American, New York

20   (1996)).

Furthermore, aplastic anemia presents a serious clinical condition as the overall mortality of all patients with aplastic anemias, in the absence of stem cell therapy, is high. Approximately 60-75% of individuals suffering from the disorder die within 12 months, in the absence of new stem cells. The overall

25   incidence of these diseases is approximately 25 new cases per million persons per year. Although it is extremely unlikely that a single pathogenic mechanism accounts for all aplastic anemias, it is clear that provision of new hematopoietic stem cells is usually sufficient to allow permanent recovery, since transplantation of patients with aplastic anemia with bone marrow obtained

from identical twins (*i.e.*, syngeneic) (Pillow, R.P., et al., 1966, *N. Engl. J. Med.* 275:94-97) or from HLA-identical siblings (*i.e.*, allogeneic) (Thomas, E.D., et al., Feb. 5, 1972, *The Lancet*, pp. 284-289) can fully correct the disease. However, some patients with aplastic anemia reject the transplanted marrow.

5   This complication is particularly common among patients who have been immunologically sensitized as a result of multiple therapeutic blood transfusions.

The current therapy available for many hematological disorders as well as the destruction of the endogenous hematopoietic cells caused by chemotherapy or

10   radiotherapy is bone marrow transplantation. However, use of bone marrow transplantation is severely restricted since it is extremely rare to have perfectly matched (genetically identical) donors, except in cases where an identical twin is available or where bone marrow cells of a patient in remission are stored in a viable frozen state. Except in such autologous cases, there is an inevitable

15   genetic mismatch of some degree, which entails serious and sometimes lethal complications. These complications are two-fold. First, the patient is usually immunologically incapacitated by drugs beforehand, in order to avoid immune rejection of the foreign bone marrow cells (host versus graft reaction). Second, when and if the donated bone marrow cells become established, they can attack

20   the patient (graft versus host disease), who is recognized as foreign. Even with closely matched family donors, these complications of partial mismatching are the cause of substantial mortality and morbidity directly due to bone marrow transplantation from a genetically different individual.

Peripheral blood has also been investigated as a source of stem cells for

25   hematopoietic reconstitution (Nothdurtt, W., et al., 1977, *Scand. J. Haematol.* 19:470-481; Sarpel, S.C., et al., 1979, *Exp. Hematol.* 7:113-120; Ragharachar, A., et al., 1983, *J. Cell. Biochem. Suppl.* 7A:78; Juttner, C.A., et al., 1985, *Brit. J. Haematol.* 61:739-745; Abrams, R.A., et al., 1983, *J. Cell. Biochem. Suppl.* 7A:53; Prummer, O., et al., 1985, *Exp. Hematol.* 13:891-898). In some studies,

9

promising results have been obtained for patients with various leukemias (Reiffers, J., et al., 1986, *Exp. Hematol.* 14:312-315; Goldman, J.M., et al., 1980, *Br. J. Haematol.* 45:223-231; Tilly, H., et al., July 19, 1986, *The Lancet,* pp. 154-155; see also To, L.B. and Juttner, C.A., 1987, *Brit. J. Haematol.* 66:

5   285-288, and references cited therein); and with lymphoma (Korbling, M., et al., 1986, *Blood* 67:529-532). Other studies using peripheral blood, however, have failed to effect reconstitution (Hershko, C., et al., 1979, *The Lancet* 1:945-947; Ochs, H.D., et al., 1981, *Pediatr. Res.* 15:601). Studies have also investigated the use of fetal liver cell transplantation (Cain, G.R., et al., 1986,

10   *Transplantation* 41:32-25; Ochs, H.D., et al., 1981, *Pediatr. Res.* 15:601; Paige, C.J., et al., 1981, *J. Exp. Med.* 153:154-165; Touraine, J.L., 1980, *Excerpta Med.* 514:277; Touraine, J.L., 1983, *Birth Defects* 19:139; see also Good, R.A., et al., 1983, *Cellular Immunol.* 82:44-45 and references cited therein) or neonatal spleen cell transplantation (Yunis, E.J., et al., 1974, *Proc. Natl. Acad. Sci.*

15   *U.S.A.* 72:4100) as stem cell sources for hematopoietic reconstitution. Cells of neonatal thymus have also been transplanted in immune reconstitution experiments (Vickery, A.C., et al., 1983, *J. Parasitol.* 69(3):478-485; Hirokawa, K., et al., 1982, *Clin. Immunol. Immunopathol.* 22:297-304).

Clearly, there is a tremendous need for methods of expanding blood cells *in*

20   *vitro* or therapies which increase the production of hematopoietic cells *in vivo.*

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2.4   WASTING SYNDROMES

Wasting syndrome is a serious clinical problem characterized by a decrease in body mass of more than 10% from baseline body weight and a disproportionate loss of body mass with respect to body fat (Weinroth et al., 1995, *Infectious*

5   *Agents and Disease* 4:76-94; Kotler and Grunfeld, 1995, *AIDS Clin. Rev.* 96:229-275). Thus, wasting is distinguished from starvation in which higher levels of body fat than body cell mass are depleted (Kotler et al., 1985, *Am J. Clin. Nutr.* 42:1255-1265; Cahill, 1970, *N. Engl. J. Med.* 282:668-675). Wasting is associated with a variety of conditions, including HIV infection (human

10   immunodeficiency virus (HIV) has been implicated in acquired immune deficiency syndrome (AIDS) (Barre-Sinoussi, F., et al., 1983, *Science* 220:868-870; Gallo, R., et al., 1984, *Science* 224:500-503)), other infectious diseases, sepsis, cancer, chronic cardiovascular disease and diarrhea (Kotler et al., 1989, *Am. J. Clin. Nutr.* 50:444-447; Heymsfield et al., 1982, *Am. J. Clin. Nutr.*

15   36:680-690). Importantly, wasting is a significant factor in the mortality of patients suffering from infections or cancer. In fact, body cell mass depletion has a linear relationship to time of survival in AIDS patients (Kotler et al., 1989, *Am. J. Clin. Nutr.* 50:444-447).

The cause of wasting syndrome in AIDS and other conditions is unclear and is

20   most likely multifactorial. Metabolic abnormalities, irregular levels of hormones and cytokines, and malabsorption have all been implicated in wasting syndrome. Not all AIDS patients suffer from wasting, suggesting that the cause of the wasting is not HIV itself. Most cases of HIV associated wasting syndrome are apparently caused by complications of AIDS, such as secondary infections

25   and gastrointestinal disease (Kotler and Grunfeld, 1995, *AIDS Clin. Rev.* 96:229-275).

Current and potential therapies for wasting syndromes include nutritional support, appetite enhancers such as dronabinol and megestrol acetate, anabolic therapies, such as growth hormone, and cytokine inhibitors. However, mixed

results have been obtained with nutritional support and appetite enhancers in that patients tended to gain only fat and not overall body mass. Administration of growth hormone, and cytokine inhibitors are still being tested and may pose a risk of side effects (Kotler and Grunfeld, 1995, *AIDS Clin. Rev.* 96:229-275;

5   Weinroth et al., 1995, *Infectious Agents and Disease* 4:76-94).

Thus, treatment of wasting is critical to the survival and well-being of patients suffering from serious diseases such as cancer and AIDS; thus, there is a need for safe and effective therapies for wasting syndrome associated with cancer, AIDS and other infectious diseases.

10 **2.5   CANCER**

A neoplasm, or tumor, is a neoplastic mass resulting from abnormal uncontrolled cell growth, which may cause swelling on the body surface, and which can be benign or malignant. Benign tumors generally remain localized. Malignant tumors are collectively termed cancers. The term "malignant"

15 generally means that the tumor can invade and destroy neighboring body structures and spread to distant sites to cause death (for review, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-122). Treatment options, such as surgery, chemotherapy and radiation treatment, are either ineffective or present serious side effects. Thus, there is

20 a need for development of new drugs for the treatment of cancer.

Kaposi's Sarcoma (KS) is a rare type of cancer, the incidence of which is greatly increased in HIV infected people (Lunardi-Iskandar, Y., et al., 1995, *Nature* 375:64-68; Friedman-Kien, A.E., et al., 1981, *J. Am. Acad. Dermatol.* 5:468-473). The tumors appear to be comprised of hyperplastic cells derived from

25 vascular endothelial cells (Nakamura, S., et al., 1988, *Science* 242:426-430; Ensoli, B., et al, 1989, *Science* 243:223-226; Salahuddin, S.Z., et al., 1988, *Science* 242:430-433; Masood, R., et al., 1994, *AIDS Res. Hum. Retroviruses*

10:969-976; Lunardi-Iskandar, Y., et al., 1995, *JNCI* 88:450-454). In some cases, neoplastic cells with chromosomal abnormalities are also present in the tumors (Lunardi-Iskandar, Y., et al., 1995, *JNCI* 87:974-981; Delli-Bovi, P., et al., 1986, *Cancer Res.* 46:6333-6338; Siegal, B., et al., 1990, *Cancer* 65:492-

5   498; Yunis, J.J., 1983, *Science* 221:227-236; Popescu, N.C., et al., 1995, *JNCI* 88:450-454). Therapies for KS include radiotherapy, α-interferon and systemic chemotherapy (Chak, L.Y., et al., 1988, *J. Clin. Oncol.* 6:863-7; Evans, L.M., et al., 1991, *J. Immunother.* 10:39-50; Kovas, J., et al., 1990, *Ann. Intern. Med.* 112:812-21; Gelmann, E.D., et al., 1987, *Am. J. Med.* 82:456-62; Gill, P.S., et

10   al., 1991, *Am. J. Med.* 90:427-33; Gill, P.S., et al., 1990; *Am. J. Clin. Oncol.* 13:315-9; Gill, P.S., et al., 1994, *AIDS* 8:1695-9). However, hematological and non-hematological toxicities limit the prolonged use of chemotherapy and α-interferon in conjunction with anti-retroviral agents commonly used in the treatment of AIDS (Kovas, J., et al., 1990, *Ann. Intern. Med.* 112:812-21; Gill,

15   P.S., et al., 1991, *Am. J. Med.* 90:427-33; Gill, P.S., et al., 1994, *AIDS* 8:1695-9). Thus, new drugs, preferably drugs compatible with AIDS therapeutics, are needed for the treatment of KS.

2.6   HUMAN CHORIONIC GONADOTROPIN

Human chorionic gonadotropin (hCG), which is required for the maintenance

20   of pregnancy, is a member of the glycoprotein hormone family. The glycoprotein hormones, which also include follicle-stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH), consist of two sub-units, α and β. These subunits are non-covalently linked to form a heterodimer, and heterodimer formation has been shown to be required for

25   receptor binding. Within a particular species, the α-subunits are identical among the glycoprotein hormones while the β-subunits differ and determine the receptor binding specificity of the particular hormone (Kornyei, J.L., et al., 1993, *Biol. Reprod.* 49:1149). The β-subunits of the glycoprotein hormones exhibit a high degree of sequence similarity within the N-terminal 114 amino

acids. LH is the most similar to hCG with 85% sequence homology within the first 114 amino acids, and both proteins bind the same receptor. hCG, however, contains a C-terminal extension not present in the other glycoprotein β-chains (Lapthorn, A.J., et al., 1994, *Science* 369:455-461).

5   From the three dimensional crystal structure of hCG, it has been determined that hCG, like the growth factors nerve growth factor (NGF), transforming growth factor-β (TGF-β) and platelet-derived growth factor-β (PDGF-β), is a cysteine-knot glycoprotein. Proteins containing such a cysteine-knot motif have at least three disulfide bridges, two of which join adjacent anti-parallel strands of the
10   peptide, thus, forming a ring, and one of which joins the peptide chain through the ring. Particular structures in the hCG β-chain include the determinant loop sequence (β93-100) which has been implicated in subunit association and the longest inter-cysteine loop (β38-57) which may play a role in receptor binding. Residues 47-53 appear to be exposed at the surface of this inter-cysteine loop
15   (Lapthorn et al., 1994, *Nature* 369:455-461).

        Previously, purified preparations of heterodimeric hCG have been shown to reduce the reverse transcriptase activity in HIV-1 infected lymphocytes and monocytes in culture (Bourinbaiar, A.S., and Nagorny, R., 1992, *FEMS Microbiology Letters* 96:27-30) and to prevent transmission of HIV from
20   lymphocytes to trophoblasts in vitro (Bourinbaiar, A.S., and Nagorny, R., 1992, *FEBS Letters* 309:82-84). Additionally, the β-subunit of hCG (β-hCG) has been demonstrated to reduce HIV production in lymphocytes at doses from 100 pg/ml to 100 μg/ml and in monocytes at doses up to approximately 10 μg/ml, with higher doses actually increasing the level of viral production in monocytes
25   (Bourinbaiar, A.S., and Lee-Huang, S., 1995, *Immunology Letters* 44:13-17). However, none of these reports disclose the potential efficacy of β-hCG peptides in HIV inhibition *in vitro* or of hCG or any portion or derivative thereof in HIV treatment or prevention *in vivo*.

14

Furthermore, doses of hCG below those necessary to induce a humoral immune response have been proposed for treatment of HIV infection based on observations of therapeutic effects of such doses on cats and cows infected with feline leukemia and bovine leukemia viruses respectively (U.S. Patent No.

5 4,880,626). This patent suggested use of the hCG dimer at very low doses (approximately 2 I.U. per treatment).

Lunardi-Iskandar et al. (1995, *Nature* 375:64-68 and PCT Application WO96/04008) reported that hCG, β-hCG, as well as a β-hCG carboxy-terminal peptides of amino acids 109-145 (SEQ ID NO:25) and 109-119 (SEQ ID NO:7)

10 are efficacious in the treatment of Kaposi's Sarcoma. However, neither reference discloses or even suggests that hCG, β-hCG or β-hCG peptides of amino acids 109-145 or 109-119 (SEQ ID NOS:7 and 25, respectively) have any viral anti-activity or that other β-hCG peptides have any therapeutic activity.

Finally, Harris (1995, *The Lancet* 346:118-119) reported that treatment with

15 hCG improved T cell counts and physical symptoms in certain HIV infected subjects.

The present invention fulfills a need for safe and effective therapies for HIV, wasting syndrome associated with AIDS, other infectious diseases and cancer, as well as methods for promoting hematopoiesis.

20 Citation of references hereinabove shall not be construed as an admission that such references are prior art to the present invention.

3 SUMMARY OF THE INVENTION

The present inventors have found that hCG preparations, β-hCG preparations, certain peptides of β-hCG, certain combinations of β-hCG peptides linked via

25 their N-termini and C-termini by peptide bond(s), and certain gel filtration chromatography fractions of commercial hCG preparations and human early (*i.e.*

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first trimester) pregnancy urine exhibit anti-viral activities, including anti-HIV activities.. In particular, hCG and β-hCG preparations and certain gel filtration chromatography fractions of commercial hCG preparations and of human early pregnancy urine, as described by way of example hereinbelow, and specific

5   peptides thereof inhibit HIV-1 replication *in vitro*, inhibit HIV-1 gene-expression in HIV-1 transgenic mice, reduce plasma virus levels in SIV infected monkeys and in AIDS patients, and increase $CD4^+$ T cells in HIV transgenic mice, SIV infected monkeys and AIDS patients. The present inventors have further found that the subjects tolerated treatment with hCG and β-hCG

10 preparations very well and that the virus did not become resistant to treatment after exposure to hCG or β-hCG. The present invention fills a tremendous need for a non-toxic, long-term treatment of HIV infection and its sequelae, ARC and AIDS.

The present invention relates to proteins having a sequence of one or more

15 portions of the β-chain of hCG (β-hCG), particularly proteins having the sequence of amino acid numbers 41-54, 45-54, 47-53, 45-57 and 109-119 (SEQ ID NOS:3-7, respectively). The present invention also relates to proteins comprising or, alternatively, consisting of, the sequence of two or more portions of β-hCG, e.g., wherein said portions are linked via their N-termini and C-

20 termini by peptide bond(s), particularly proteins having the sequence of amino acid numbers 45-57 (SEQ ID NO:6) linked via a peptide bond at the C-terminus to the N-terminus of a peptide of amino acid numbers 109-119 (SEQ ID NO:7) or linked at the N-terminus to the C-terminus of a peptide of amino acid numbers 110-119 (SEQ ID NO:27); or a peptide of amino acid numbers 47-57

25 (SEQ ID NO:28) linked by a peptide bond at the C-terminus to the N-terminus of a peptide of amino acid numbers 108-119 (SEQ ID NO:29) of β-hCG as depicted in Figure 8 (a portion of SEQ ID NO:2), *i.e.* the peptides denoted 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). The present invention also relates to certain fractions (*i.e.*

30 components of a source of hCG or β-hCG isolated away from other components

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in the source of hCG or β-hCG by a separation technique known in the art) of any source of hCG or β-hCG, such as commercial hCG preparations and human (preferably early, *i.e.*, first trimester) pregnancy urine, which fractions have anti-HIV and/or anti-Kaposi's Sarcoma activity.

5   The present invention further relates to therapeutic methods and compositions for treatment and prevention of diseases and disorders associated with HIV infection based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG (preferably a source of native hCG or β-hCG, *i.e.* a source of naturally occurring hCG or β-

10   hCG, not recombinantly produced hCG or β-hCG) and therapeutically and prophylactically effective proteins containing a sequence of one or more portions (*i.e.*, a fusion protein comprising more than one β-hCG peptide sequence either as non-contiguous or contiguous sequences, *e.g.*, having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another

15   β-hCG peptide) of β-hCG, and related derivatives and analogs. The invention provides for treatment and prevention of HIV infection by administration of a therapeutic compound of the invention. The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG, therapeutically and prophylactically

20   effective peptides having a sequence of a one or more portions of β-hCG, modified derivatives of hCG, β-hCG and β-hCG peptides, and nucleic acids encoding β-hCG and therapeutically and prophylactically effective peptides having a sequence of one or more portions of β-hCG, and derivatives and analogs of the foregoing. The invention also provides *in vitro* and *in vivo*

25   assays for assessing the efficacy of therapeutics of the invention for treatment or prevention of HIV. The invention also provides pharmaceutical compositions and methods of administration of therapeutics of the invention for treatment or prevention of HIV infection.

The present invention further relates to therapeutic methods and compositions having anti-wasting activity for treatment and prevention of wasting syndromes based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG and

5   therapeutically and prophylactically effective proteins containing a sequence of a portion or portions (i.e., a fusion protein comprising more than one β-hCG peptide sequence, e.g., having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide) of β-hCG, and related derivatives and analogs. The invention provides for treatment and prevention

10   of wasting syndromes by administration of a therapeutic compound of the invention. The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG (preferably a source of native hCG or native β-hCG, i.e. a source of naturally occurring hCG or β-hCG and not recombinantly produced hCG or β-

15   hCG), therapeutically and prophylactically effective peptides having a sequence of a portion or portions of β-hCG (i.e. a fusion protein comprising more than one β-hCG peptide sequence either as non-contiguous or contiguous sequences, e.g. having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide), modified derivatives of hCG, β-hCG and β-hCG

20   peptides, and nucleic acids encoding β-hCG and therapeutically and prophylactically effective peptides having a sequence of a portion or portions of β-hCG, and derivatives and analogs of the foregoing.

The present invention also relates to the use of certain fractions (i.e. components of a source of hCG or β-hCG isolated away from other components

25   in the source of hCG or β-hCG by a separation technique known in the art) of any source of hCG or β-hCG, such as commercial hCG preparations and human (preferably early, i.e., first trimester) pregnancy urine, which fractions have anti-HIV, anti-cancer activity (such as anti-Keposi's Sarcoma activity), anti-wasting activity and/or pro-hematopoeitic activity.

The invention also provides *in vitro* and *in vivo* assays for assessing the efficacy of therapeutics of the invention for treatment or prevention of HIV infection, cancer, and/or wasting syndromes.

The invention also provides pharmaceutical compositions and methods of
5   administration of Therapeutics of the invention for treatment.

The present invention also relates to therapeutic methods and compositions for treatment and prevention of diseases and disorders in which an increase in one or more types of hematopoietic cells is desirable. The therapeutic compounds of the invention are hCG and β-hCG preparations, therapeutically and
10   prophylactically effective fractions of a source of native hCG or native β-hCG and therapeutically and prophylactically effective proteins containing a sequence of one or more portions (*i.e.*, a fusion protein comprising more than one β-hCG peptide sequence either as non-contigous or contiguous sequences, *e.g.*, having an amino acid sequence of one β-hCG peptide linked via a peptide
15   bond to another β-hCG peptide) of β-hCG, and related derivatives and analogs. The present invention also relates to use of certain fractions (*i.e.* components of a source of hCG or β-hCG (preferably native hCG or β-hCG, *i.e.* not recombinantly produced) isolated away from other components in the source of hCG or β-hCG by a separation technique known in the art) of any source of hCG
20   or β-hCG, such as commercial hCG preparations and human (preferably early, *i.e.*, first trimester) pregnancy urine, which fractions have anti-HIV, anti- cancer activity (such as anti-Kaposi's Sarcoma activity) anti-wasting activity and/or pro-hematopoietic activity.

The invention provides for treatment and prevention of diseases and disorders
25   (*e.g.*, involving hematopoietic cell deficiencies) by administration either of a therapeutic compound of the invention or of hematopoietic cells, the numbers of which have been increased *in vitro* by contact with a therapeutic compound of the invention. The invention thus also provides *in vitro* methods of

expanding hematopoietic cells. The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, therapeutically and prophylactically effective peptides having a sequence of one or more portions of β-hCG,

5   modified derivatives of hCG, β-hCG and β-hCG peptides, and nucleic acids encoding β-hCG and therapeutically and prophylactically effective peptides having a sequence of one or more portions of β-hCG, and derivatives and analogs of the foregoing.

In a specific embodiment, gene therapy methods are provided using hCG and

10   β-hCG preparations, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG and therapeutically and prophylactically effective proteins containing a sequence of one or more portions of β-hCG, and related derivatives and analogs to induce proliferation of hematopoietic progenitor or stem cells into which cells a nucleic acid of interest is introduced either before

15   or after proliferation. The proliferation induced by the methods of the invention can be with or without concomitant hematopoietic cell differentiation, and, in a preferred embodiment, is proliferation followed by differentiation of the cells.

The present invention further relates to therapeutic methods and compositions

20   for treatment and prevention of cancers based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG and therapeutically and prophylactically effective proteins containing a sequence of one or more portions (i.e., a fusion protein comprising more than one β-hCG peptide sequence either as non-contiguous or contiguous

25   sequences, e.g., having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide) of β-hCG, and related derivatives and analogs. The invention provides for treatment and prevention of cancers by administration of a therapeutic compound of the invention. The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and

20

prophylactically effective fractions of a source of native hCG or native β-hCG, therapeutically and prophylactically effective peptides having a sequence of one or more portions of β-hCG, modified derivatives of hCG, β-hCG and β-hCG peptides, and nucleic acids encoding β-hCG and therapeutically and

5 prophylactically effective peptides having a sequence of one or more portions of β-hCG, and derivatives and analogs of the foregoing.

The present invention also relates to certain fractions (*i.e.* components of a source of hCG or β-hCG isolated away from other components in the source of hCG or β-hCG by any separation technique known in the art) of any source of

10 hCG or β-hCG, such as commercial hCG preparations and human (preferably early, *i.e.*, first trimester) pregnancy urine, which fractions have anti-HIV and or anti-Kaposi's Sarcoma activity. The invention also provides *in vitro* and *in vivo* assays for assessing the efficacy of therapeutics of the invention for treatment or prevention of cancers.

15 The invention also provides pharmaceutical compositions and methods of administration of Therapeutics of the invention for treatment.

The invention also provides methods of administration and pharmaceutical compositions containing a Therapeutic of the invention.

3.1 DEFINITIONS

20 As used herein, the following terms shall have the meaning indicated.

AIDS = Acquired Immune Deficiency Syndrome

ARC = AIDS-Related Complex

21

BFU-E = burst forming unit-erythroid. A hematopoietic progenitor cell which is capable of producing a colony of erythroid progeny cells in semi-solid medium.

CFU = colony forming unit. A cell which is capable of producing a colony of progeny cells in semi-solid medium.

5

CFU-GEMM = colony forming unit-granulocyte, erythrocyte, monocyte/macrophage, megakaryocyte. A multipotential hematopoietic progenitor cell which is capable of producing a colony composed of granulocyte, erythrocyte, monocyte/macrophage, and megakaryocyte progeny in semi-solid medium.

10

CFU-GM = colony forming unit-granulocyte, macrophage. A hematopoietic progenitor cell which is capable of producing a colony composed of granulocyte and macrophage progeny in semi-solid medium.

15

CFU-MK = colony forming unit-megakaryocyte. A hematopoietic progenitor cell which is capable of producing a colony composed of megakaryocyte progeny in semi-solid medium.

CFU-S = colony forming unit-spleen. A multipotential stem cell with self-renewal capacity, which, upon inoculation into a lethally irradiated mouse, is capable of producing a colony (module) on the spleen. CFU-S is not a marrow-repopulating cell; it is a less primitive stem cell which does not provide long-term engraftment in an animal.

20

CSF = colony stimulating factor

25

Epo   =   erythropoietin

FBS   =   fetal bovine serum. Also known as fetal calf serum.

G-CSF   =   granulocyte colony stimulating factor

GM-CSF =   granulocyte-macrophage colony stimulating factor

5   HCG =   Human Chorionic Gonadotropin

KS   =   Kaposi's Sarcoma

OI   =   Opportunistic Infection

ITP   =   Idiopathic thrombocytopenic purpura (a severe platelet deficiency)

10   PB   =   peripheral blood

PBMC =   Peripheral Blood Mononuclear Cell

4   DESCRIPTION OF THE FIGURES

Figures 1A-E. Effects of an hCG preparation, APL™ (Wyeth-Ayerst), β-hCG peptides and certain fractions of hCG APL™ and early pregnancy urine on

15   weight and HIV-1 gene expression in HIV-1 transgenic mice. (A) and (C) Weight change in grams in individual HIV-1 transgenic mice after treatment from day 1 to day 10 postpartum is represented as a bar graph with open bars representing the weight at day 1 and solid bars representing the weight at day 10. (B), (D) and (E) Suppression of HIV-1 gene expression in transgenic mice.

20   The bar graph presents the level of expression in pixels, as determined by chemiluminescence assay of the HIV genes env, tat, rev, and nef in the

individual HIV transgenic mice. For (B) and (D), the black bars represent tat expression, the striped bars represent rev expression, the lightly stippled bars represent env expression, the open bars represent nef expression. For (E), the striped bars represent env expression, the solid bars represent rev expression, and the open bars represent nef expression. In (A) and (B), bars 1-3 represent untreated control transgenic mice; bars 4-6 represent mice whose mothers received (subjects were administered through the mothers' milk) 200 µg circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) per day; bars 7-9 represent mice whose mothers received 300 IU per day hCG-APL™; and bars 9-11 represent mice whose mothers received 200 µg per day of the fused β-hCG peptide 45-57::109-119 (SEQ ID NO:30). In (C) and (D), the bars labeled "untreated" represent mice receiving only PBS; and the bars labeled "treated" or "treated with HAFc" represent mice administered 300 IU per day of hCG APL™. In (E), the set of bars labeled "1" represents mice treated with PBS alone; "2" represents mice treated with 100 µg β-hCG core peptide per day; "3" represents mice treated with 100 µg per day α-hCG; "4" represents mice treated with 200 µl per day of fraction 61 of the hCG APL™ fractionation; and "5" represents mice treated with 200 µl per day of fraction 65 of the early pregnancy urine fractionation.

Figures 2A-D. Effects of an hCG preparation on indicators of SIV infection in SIV-infected macaques. SIV was given intravenously at a dose of $10^{4.5}$ TCID$_{50}$ per ml. (A) SIV titer was monitored over time in months by quantifying the p27 gag protein (Organon Teknika assay) as nanograms (ng) of p27/ml of plasma from the plasma of the SIV infected macaques. Treated SIV-infected macaques (indicated as Rx) were given hCG APL™, 3000 IU, 2x weekly. Plasma levels of p27 gag in these treated monkeys are indicated on the graph by lines with diamonds, number (#) signs or filled circles. Results with the untreated SIV-infected macaques (indicated UnRx) are indicated by the lines with either stars or triangles. (B) CD4$^+$ T cell levels were determined in cells/mm^3 in SIV-infected macaques either treated with hCG or untreated over time in

24

months. Results from the SIV-infected monkeys treated with hCG (APL™) (Rx) are indicated by lines with diamonds, number (#) signs or filled circles, while results with the untreated monkeys (UnRx) are indicated by lines with stars or triangles. (C) Change in weight in kilograms (kg) was monitored in treated and

5 untreated SIV-infected monkeys over time in months. Weight changes in the SIV-infected monkeys treated with hCG (APL™) (Rx) are indicated by lines with diamonds, # signs or filled circles, while results in the untreated monkeys (UnRx) are indicated by lines with stars or triangles. (D) Levels of CD4$^+$ T cells were monitored in normal uninfected monkeys either treated with hCG (APL™)

10 or untreated over time in months. CD4$^+$ T cell levels in the untreated monkeys are indicated by lines with sun-like figures or squares, and the results in the treated monkeys are indicated by lines with pentagonal figures or with filled inverted triangles.

Figures 3A-J. Effects of administration of hCG preparations on HIV-1 viral load

15 and CD4$^+$ T cell levels in individual patients in the clinical study described herein. Figures A and B are data from patient PHOJ, C and D from patient PG1, E and F from patient PG3, G and H from patient PHVE, and I and J from patient PG17. In panels A, C, E, G and I, viral load and CD4$^+$ T Cell counts are plotted over time (in months). Viral load (measured by RT-PCR in panels A and G and

20 by the Roche Amplicor test in panels C, E and I) is plotted as the logarithm of the viral load (represented by line with "X" data points). The CD4$^+$ T Cell levels are plotted as CD4$^+$ T Cells/ml (represented by line with triangle data points). Panels B, D, F, H, and J plot the dosage of hCG in IU (X 1000) per week over time in months, with the timing of other therapies indicated above

25 the graph with a thick arrow.

Figures 4A-D. Effects of hCG preparations and peptides on HIV replication *in vitro*. (A and B) These graphs depict the concentration dependence of inhibition of HIV-1 IIIB infection of CD4$^+$ T cells or total PBMCs from peripheral blood of normal donors (infection is expressed in nanograms (ng) of

p24/ml plasma) as a function of nmol per ml α-hCG subunit, β-hCG peptide or α-hCG peptide over a concentration of 0.05 to 50 nmol/ml. Graphs present data on infection of (A) CD4^{+} T cells infected by HIV-1 IIIB and (B) PBMCs infected with HIV-1 IIIB. In both graphs, results with α-hCG subunit are represented by

5   lines with diamonds, results with the β-hCG peptide 6-16 by lines with squares, results with the β-hCG peptide 109-119 by lines with stars, results with the circularized β-hCG peptide 44-57 (with cysteine substituted for the amino acid at position 44) (SEQ ID NO:26) by lines with triangles, and results with the α-hCG peptide 88-92 by lines with inverted triangles. (C and D) These graphs

10   depict data on the effect of α-hCG, hCG peptides and commercial hCG preparations on the infection of primary macrophages by HIV-1 Ba-L. (C) Effect of different concentrations of hCG α subunits and various hCG peptides (0.05 to 50 nmol/ml) on infection in peripheral blood macrophages from a normal donor infected with HIV-1 Ba-L. The results are averages of triplicate samples

15   with less than 15% variation. Results with α-hCG subunit are represented by lines with diamonds, results with the β-hCG peptide 6-16 by lines with squares, results with the β-hCG peptide 109-119 (SEQ ID NO:7) by lines with stars, results with the circularized β-hCG peptide 44-57 (with cysteine substituted for the amino acid at position 44) (SEQ ID NO:26) by lines with triangles, and

20   results with the α-hCG peptide 88-92 by lines with inverted triangles. (D) A comparison of the inhibition of HIV Ba-L infection of macrophages by different commercial native hCG preparations (APL™, Wyeth Ayerst; STERIS™, Steris; PREGNYL™, Organon) and by purified hCG (CR127) over a concentration range of 0.05 IU to 1,000 IU/ml. Results are shown at day 10. Results with APL™

25   hCG are indicated by a line with inverted triangles, results with STERIS™ hCG are indicated by a line with circles, results with PREGNYL™ hCG are indicated by a line with squares, and results with hCG-CR127 are indicated by a line with X's.

26

Figures 5A-C. Effect of preparations of hCG on HIV-LTR activity. (A) The CAT activity of cells containing the HIV-LTR construct and treated with hCG concentrations of 0 IU/ml, 100 IU/ml, 250 IU/ml and 500 IU/ml was calculated relative to the untreated control. (B) Relative CAT activity of the unrelated

5   SV40 promoter in response to hCG at 0 IU/ml, 250 IU/ml and 500 IU/ml was similarly calculated. Data in both A and B represents the mean +/- S.E.M. of 3 to 7 independent experiments and is presented as a bar graph. The different hCG concentrations are indicated in both A and B as depicted below:

```
0       =
100 IU/ml  =
250 IU/ml  =
500 IU/ml  =
```
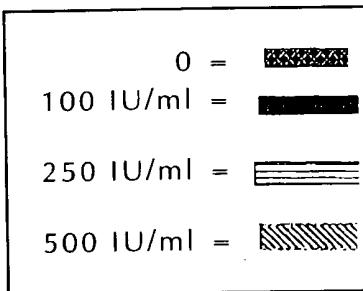
10

(C) The viability of Hut 78 cells in the presence of hCG at 0 IU/ml, 250 IU/ml and 500 IU/ml under the conditions used in the transient expression assays shown in Figures A and B was determined using a coulter cell counter. Results are presented as a bar graph in terms of cell number x 10,000 and the hCG

15   concentrations are indicated as shown below:

```
0       =
100 IU/ml  =
250 IU/ml  =
500 IU/ml  =
```

27
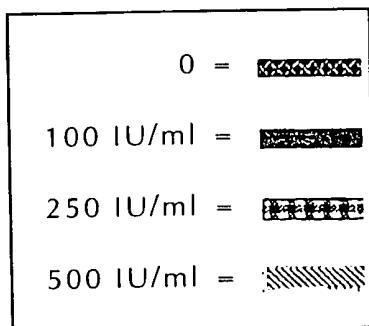
Figures 6A-H. Effect of hCG preparations and peptides on KS colony growth *in vitro* and KS tumors *in vivo*. (A) Comparison of the anti-KS *in vitro* effects (tumor cell killing) of purified hCG and β-hCG peptides in KS clonogenic assays using KS Y-12 and KS Y-12 "SKL"18 cells depicted in a bar graph in terms of

5   number of colonies. The results are averages of 3 sets of results with less than 10% variation and are representative of multiple experiments. Results with no hCG or hCG peptides are represented by open bars, the results with the β-hCG peptide of amino acids 109-119 (SEQ ID NO:7) are represented by stippled bars, the results with the β-hCG peptide of amino acids 109-145 (SEQ ID NO:25) are

10   represented by the bars with horizontal stripes, the results with the circular β-hCG peptide of amino acids 44-57 (SEQ ID NO:26) where the amino acid at position 44 is a cysteine are represented by the bars with diagonal stripes, and the results with the highly purified hCG preparation, CR 127, are represented by solid bars. (B-H) Thin sections of KS tumors induced in nude mice by

15   inoculation with 10^6 neoplastic KS Y-1 cells. (B) Thin section of tumors from mice that were not treated with hCG or hCG subunits or peptides (C) Thin section of a tumor from a mouse after treatment with crude hCG APL™ (100 IU) subcutaneously daily for 7 days. (D) Thin section of a tumor from a mouse treated with the β-hCG peptide of amino acids 45-57 (SEQ ID NO:6), 10

20   μg/ml/daily (6.7 nmoles) for 5 days. (E) Thin section of a tumor from a mouse after 5 days of treatment with the circularized β-hCG peptide 44-57 where cysteine has been substituted at position 44 (SEQ ID NO:26), at 10 μg per day. (F) This panel shows the thin tissue section of KS tumor from AIDS-KS patients treated with 1 ml of diluent alone shows less than 2% cell death as detected by

25   specific apoptosis in situ immunostaining. (G) Thin tissue section of KS tumor from an AIDS-KS patient after hCG preparation therapy of intralesional injections of 2000 IU, 3 times weekly for 2-3 weeks, shows evidence of apoptosis in all cells. (H) Thin tissue section of KS tumor from an AIDS-KS patient after hCG preparation therapy, 500 IU, 3 times weekly for 3 weeks.

28

Figures 7A-C. These bar graphs demonstrate the effects of hCG preparations and peptides on hematopoiesis *in vitro*. (A) This bar graph depicts results of colony assays in terms of number of colonies for CFU-MIX (colony forming units of megakaryocytes, erythrocytes, granulocytes and monocytes). (B) This bar graph

5   presents data from colony assays for BFU-e (Burst forming units of erythrocytes) in terms of number of colonies. (C) This bar graph presents results from colony assays of CFU-GM (colony forming units of granulo-macrophages) in terms of number of colonies. For all three graphs, results are shown for cells isolated from cord blood ("cord") and bone marrow ("marrow"). The results are averages

10   of 3 sets of results with less than 10% variation and are representative of multiple experiments. The results from no treatment are indicated by open bars; the results with α-hCG are represented by solid bars; the results with APL™ hCG (hCGapl) are represented by bars with a lattice pattern; the results with native β-hCG preparation (NbhCG) are represented by cross-hatched bars; the results

15   with the highly purified hCG preparation (CR127) are represented by open bars; the results with the β-hCG peptide of amino acids 109-119 (SEQ ID NO:7) (b109-119) are shown by the diagonally stippled bars; the results with the β-hCG peptide of amino acids 45-57 (SEQ ID NO:6) (b45-57) are shown by the bars with the diamonds; the results with the circularized β-hCG peptide of

20   amino acids 44-57 with cysteine substituted for the amino acid at position 44 (SEQ ID NO:26) (b45-57c) are represented by the diagonally striped bars; and the results with the mixture of scrambled β-hCG peptides of amino acids 45-57 and 109-119 (bmix45+109) are represented by the vertically striped bars.

Figure 8. Nucleotide (SEQ 1D NO:1) and amino acid (SEQ 1D NO:2) sequence

25   of β-hCG.

Figures 9A and B. Schematic depiction of the structures of (A) the linear peptide of amino acids 45-57 (SEQ ID NO:6) of the β-hCG sequence depicted in Figure 8 (SEQ ID NO:2) where the amino acid residues at positions 47 and 51 are substituted by a branch made up of diaminobutyric acid peptide bonded

to proline, and (B) the circularized peptide of amino acids 44-57 (SEQ ID NO:26) with valine at position 44 substituted with cysteine, which protein is circularized via a disulfide bond between its amino- and carboxy-terminal cysteines. In both A and B, amino acids are represented by their three letter
5   amino acid code, except for the branched residues and the terminal cysteines, for which the structure is depicted.

Figures 10A-F. These graphs depict results from the fractionation by Superdex 200 gel filtration of a commercial hCG preparation APL™ (Wyeth Ayerst) and early pregnancy urine. (A) and (D). These graphs depicts the relative amount
10   of protein in mg/ml in each fraction identified by fraction number in the hCG APL™ fractionation (A) and early pregnancy urine fractionation (D).   The fractions containing the hCG dimer and β-core protein are identified with arrows and the labels "hCG" and "β-core" respectively. (B) and (E). These graphs present the percent inhibition of growth of cultured KS cells by the
15   individual fractions from the hCG APL™ (B) and early pregnancy urine (E) using KS cell clonogenic assays. The results are plotted as percent inhibition versus fraction number. (C) and (F). These graphs depict the effect of the hCG APL™ (C) and early pregnancy urine (F) fractions on HIV replication in vitro. Specifically, this graph presents data on the percentage inhibition of HIV-1 IIIB
20   viral infection of PBMCs as a function of fraction number.

Figure 11. Effect of hCG preparations, hCG and early pregnancy urine fractions, and β-hCG peptides on KS cell growth in vitro. Results for clonogenic assays using the cell lines KS Y-1 (bars labeled "KS Y-1") and KS SLK (labeled "KS SLK") are presented on a bar graph as percent inhibition of KS Colony Growth. Bars
25   1-4 represent cells treated with 200 IU/ml of the commercial hCG preparations hCG APL™, hCG CG10 (Sigma), hCG PROFASI™, and hCG PREGNYL™, respectively; bar 5 represents treatment with 50 μg/ml β-hCG core protein; bar 6, 50 μg/ml native β-hCG; bar 7, 50μg/ml native α-hCG; bar 8, 200 IU/ml highly purified hCG preparation CR 127; bar 9, 50 μg/ml recombinantly produced hCG

(Sigma); bars 10 and 11, 50-100 µl/ml of fractions 65 and 76, respectively, of the early pregnancy urine fractionation; bars 12 and 13, 50-100 µl/ml of fractions 65 and 76, respectively, of the hCG APL™ fractionation; and bar 14, 100 µg/ml of the circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26).

Figures 12A-C. These bar graphs demonstrate the effects of hCG preparations, fractions and peptides on hematopoiesis *in vitro*. (A) Results of colony assays in terms of percent increase of hematopoiesis for CFU-GEMM (colony forming units of megakaryocytes, erythrocytes, granulocytes and monocytes). (B) Data from colony assays for BFU-e (Burst forming units of erythrocytes) in terms of percent increase of hematopoiesis. (C) Results from colony assays of CFU-GM (colony forming units of granulo-macrophages) in terms of percent increase of hematopoiesis. In all three graphs, bar 1 represents results from treatment with PBS alone; bar 2, the results with 100 µg/ml α-hCG; bar 3, with 200 IU/ml APL™ hCG; bar 4, 200 IU/ml of the highly purified hCG preparation CR 127; bar 5, 100 µg/ml native β-hCG preparation; bar 6, 100 µg/ml of the circularized β-hCG peptide of amino acids 44-57 with cysteine substituted for the amino acid at position 44 (SEQ ID NO:26); bar 7, 100 µl/ml of fraction 65 of the hCG APL™ fractionation; bars 8 and 9, 100 µl/ml of fractions 65 and 26, respectively, of the early pregnancy urine fractionation; and bar 10, 100 µg/ml of the β-hCG core protein.

Figure 13. Effect of treatment with hCG commercial preparation and early pregnancy urine fractions on KS tumors in mice. Results are plotted as percent inhibition of tumor size as compared to control tumors. The open bar represents mice treated with PBS alone; the solid bar with 100 IU per day of hCG APL™; diagonally striped bars with 200 µl per day of fractions 26, 76, 65, and 82 (as labeled on top of the bars) of the early pregnancy urine fractionation ("HAF-UF#"); and open bars with 200 µl per day of fractions 62, 65, 74, 76 and

31

35 (as labeled on top of the bars) of the hCG APL™ fractionation ("HAF-CF#").

Figure 14. Effect of hCG preparations, peptides and fractions on survival of HIV-1 transgenic mice. Data is plotted on a bar graph as "% Survival of Tg26

5   mice" 10 days after birth, each bar representing 4 mice. None of the untreated mice survived. Bar labeled "ahCG" represents mice treated with 200 μg per day native α-hCG; bar labeled "CR127", with 300 IU per day of the highly purified hCG preparation CR 127; bar 1, 200 μg per day native β-hCG; bar 2, 300 IU per day hCG APL™; bar labeled "rβhCG", with 200 μg per day recombinant β-hCG;

10   bar 3, with 50 μg per day LH (leutinizing hormone); bar 4, with 200 μl per day fraction 65 of the hCG APL™ fractionation; bars 5 and 6, 200 μl per day fractions 65 and 76, respectively, of the early pregnancy urine fractionation; bars labeled "HAF-UF#26 and "HAF-CF#26", 200 μl per day fraction 26 from the early pregnancy urine and hCG APL™ fractionations, respectively; bar labelled

15   "b-core", 50 μg per day β-hCG core peptide; and bar 7, with 300 μg per day circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26).

Figures 15A-C. Graphs of change in viral load and CD4$^+$ T cell levels with hCG therapy. (A) The change in viral load is plotted as the logarithm of viral load

20   after therapy ("Logload") as a function of viral load before therapy ("Baselog"). (B) The change in CD4$^+$ T cell levels is plotted as CD4$^+$ T cell levels after therapy (in CD4$^+$ T cells/ml) ("CD4") as a function of CD4$^+$ T cell levels before therapy (in CD4$^+$ T cells/ml) ("CD4Base"). (C) Plot of linear regression analysis of the change in viral load ("vlchange") as a function of weekly dose

25   of hCG in IU ("HCGIU"). For all three panels, data points for patients on hCG therapy as well as non-protease and protease inhibitors are represented by open triangles, those on hCG therapy and non-protease inhibitors by open diamonds, and those on hCG alone by solid circles.

Figures 16A and B.   (A) Plot of protein concentration (as mAUFS, milli absorbance units, at 280 nm) as a function of the fraction number of the hCG APL™ preparation Superdex 200 fractionation.   (B)   Plot of protein concentration (as mAUFS, milli absorbance units, at 280 nm) of molecular weight markers of 670 kD, 158 kD, 44 kD, 17 kD and 1.3 kD (as indicated above the plot) as a function of fraction number of a Superdex 200 column run under the same conditions as the fractionation plotted in panel A.

Figures 17A-E.   Mass spectrometry profiles of fractions 61, 63, 64, 65, and 67 in panels A-E, respectively.

Figures 18 A-H.   Visualization by confocal microscopy of apoptosis of prostate cancer cells treated with hCG and hCG related preparations for 48 hours and stained with both actin monoclonal antibody labeled with FITC and propidium iodine.   Panel A shows a confocal micrograph of cultured prostate tumor cells untreated; Panel B, prostate tumor cells treated with 200 IU hCG APL™; Panel C, prostate tumor cells treated with 200 µg of the circularized β-hCG peptide of amino acids 44-57 with cysteine substituted at position 44 (SEQ ID NO:26); Panel D, prostate tumor cells treated with 100 µl of fraction number 64 of the early pregnancy urine; Panels E-H are controls treated with PBS alone.

Figure 19.   Results of clonogenic assays on prostate tumor cells with hCG preparations and fractions of the hCG APL™ preparations.   Results are plotted both as number of colonies (bars) and percent inhibition of colony formation (line with solid diamonds) for each test substance.   "PBS" are cells treated with PBS alone; "apl100" and "apl200" are cells treated with 100 IU and 200 IU of the hCG APL™ preparation, respectively; the numbers are cells treated with those fraction numbers of the fractionation of the hCG APL™ preparation; "alpha" are those cells treated with a native α-hCG preparation; and "apl-d" are

those cells treated with the hCG APL™ diluent alone (*i.e.*, a control without hCG).

Figures 20A-C. Photographs showing the levels of apoptosis in tumor cells in nude mice in response to treatment with hCG preparations or β-hCG peptides.
5   Panel A presents tumor cells from untreated mice. Panel B presents tumor cells from mice treated with 100 μg/day of the circularized β-hCG peptide of amino acids 44-57 with cysteine substituted at position 44 (SEQ ID NO:26). Panel C presents tumor cells from mice treated with 100 IU per day of hCG APL™.

Figure 21. Bar graph depicting the percent of dead lung cancer cells as
10   measured by Trypan Blue assay in response to hCG preparations, hCG fractions, and β-hCG peptides. Treatments are indicated as follows: "neg/pbs100" is control treated with PBS alone; "apl/200" and "apl/500" were treated with 200 IU and 500 IU, respectively, of the hCG APL™ preparation; "sata2/200" and "sata2/300" were treated with 200 μg/ml and 300 μg/ml, respectively, of the
15   circularized β-hCG peptide of amino acids 44-57, with cysteine substituted for position 44 (SEQ ID NO:26); "uf-60/200", "uf-64/200", "uf-74/200", "uf-23/200" and "uf-80/200" were treated with 200 μg/ml of the early pregnancy urine SUPERDEX™ 200 fractions 60, 64, 74, 23, and 80, respectively; "apl-64/200", "apl-65/200", "apl-67/200", "apl-72/200", "apl-74/200" and "apl-75/200" were
20   treated with 200 μg/ml of the hCG APL™ SUPERDEX™ 200 fractions 64, 65, 67, 72, 74, and 75, respectively.

Figures 22A-C. Results of clonogenic assays on lung tumor cells with hCG preparations, fractions of hCG preparations, and β-hCG peptides. Results are plotted both as number of colonies (bars) and percent inhibition of colony
25   formation (line with solid diamonds) for each test substance. (A) Treatments are indicated as follows: "100ul PBS" is control treated with PBS alone; "APL100" and "APL200" were treated with 100 IU and 200 IU, respectively, of the hCG APL™ preparation; "SATA2100" and "SATA2200" were treated with 100 μg/ml

and 200 µg/ml, respectively, of the circularized β-hCG peptide of amino acids 44-57, with cysteine substituted for position 44 (SEQ ID NO:26); "uf-60/200", "uf-64/200", and "uf-74/200" were treated with 200 µg/ml of the early pregnancy urine SUPERDEX™ 200 fractions 60, 64, and 74, respectively; "apl-

5   60/200", "apl-64/200" and "apl-74/200" were treated with 200 µg/ml of the hCG APL™ SUPERDEX™ 200 fractions 60, 64, and 74, respectively. (B) Results after 6 days of treatment. Treatments are indicated as follows: "100ul PBS" is control treated with PBS alone; "APL100" and was treated with 100 IU of the hCG APL™ preparation; "SATA1100" was treated with 100 µg/ml of the β-hCG peptide of

10   amino acids 45-57 (SEQ ID NO: 6); "SATA2100" was treated with 100 µg/ml of the circularized β-hCG peptide of amino acids 44-57, with cysteine substituted for position 44 (SEQ ID NO:26); and "SATAB100" was treated with 100 µg/ml of the fused β-hCG peptide 45-57::109-119 (SEQ ID NO:30). (C) Results after 5 days of treatment. Treatments are indicated as follows: "100ul PBS" is control

15   treated with PBS alone; "APL100" and "APL200" were treated with 100 IU and 200 IU, respectively, of the hCG APL™ preparation; "SATA2100" and "SATA2200" were treated with 100 µg/ml and 200 µg/ml, respectively, of the circularized β-hCG peptide of amino acids 44-57, with cysteine substituted for position 44 (SEQ ID NO:26); "uf-60/200", "uf-64/200", and "uf-74/200" were

20   treated with 200 µg/ml of the early pregnancy urine SUPERDEX™ 200 fractions 60, 64, and 74, respectively; "apl-60/200", "apl-64/200" and "apl-74/200" were treated with 200 µg/ml of the hCG APL™ SUPERDEX™ 200 fractions 60, 64, and 74, respectively.

Figures 24 A-H. Visualization by confocal microscopy of apoptosis of lung

25   cancer cells treated with hCG and hCG related preparations for 48 hours and stained with both actin monoclonal antibody labeled with FITC and propidium iodine. Panel A shows a confocal micrograph of cultured lung tumor cells untreated; Panel B, lung tumor cells treated with 200 IU hCG APL™; Panel C, lung tumor cells treated with 200 µg of the circularized β-hCG peptide of amino

30   acids 44-57 with cysteine substituted at position 44 (SEQ ID NO:26); Panel D,

lung tumor cells treated with fraction number 64 of the early pregnancy urine; Panels E-H are controls treated with PBS alone.

Figures 23 A-I. Visualization by confocal microscopy of apoptosis of kidney (renal) cancer cells treated with hCG APL™ preparation and stained with both
5   actin monoclonal antibody labeled with FITC and propidium iodine. Panels A-C show a confocal micrograph of cultured kidney cancer cells treated with PBS alone; Panels D-F, cultured kidney cancer cells treated for 48 hours with 100 IU hCG APL™; Panels G-I, cultured kidney cancer cells treated with 300 IU hCG APL™ for 48 hours.

10   ## 5   DETAILED DESCRIPTION OF THE INVENTION
The present invention relates to proteins (including peptides) containing a sequence of one or more portions of β-hCG (β-hCG peptides) that are effective at inhibiting HIV replication and/or infection *in vitro* or *in vivo*, decreasing viral load, and/or treating or preventing disorders associated with HIV infection.
15   In specific embodiments, the invention provides an isolated protein, comprising, or alternatively, consisting of an amino acid sequence amino acid numbers 41-54, 45-54, 47-53 or 45-57 (SEQ ID NOS:3-6, respectively) of the β-hCG sequence depicted in Figure 8 (a portion of SEQ ID NO:2), particularly to an isolated protein or peptide comprising an amino acid sequence consisting of
20   amino acids 45-57 (SEQ ID NO:5) of Figure 8 (a portion of SEQ ID NO:2). The invention also provides isolated proteins comprising or, alternatively, consisting of, the amino acid sequence of two or more portions (preferably non-naturally contiguous portions) of β-hCG, *e.g.*, wherein such portions are linked at the C-termini and N-termini via peptide bond(s). Specifically, the invention provides
25   isolated proteins having an amino acid sequence of β-hCG amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids β-hCG 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of β-hCG amino acids 110-119 (SEQ ID

NO:27); or an isolated protein of β-hCG amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids β-hCG 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2), *i.e.*, the fused peptides denoted 45-57::109-119,

5 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively).

The present invention also relates to certain fractions (*i.e.* components of a source of native hCG or native β-hCG isolated away from other components in the source of native hCG or native β-hCG by a separation technique known in the art), particularly fractions from gel filtration sizing chromatography, of a

10 source of native hCG or β-hCG, such as commercial preparations of hCG and human (preferably early, *i.e.* first trimester) pregnancy urine, that have anti-HIV and/or anti-KS activity. Native hCG or native β-hCG refers to naturally occurring hCG or β-hCG, *i.e.* not recombinantly produced.

The present invention further relates to therapeutic methods and compositions

15 for treatment and prevention of disorders associated with HIV infection based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, and therapeutically and prophylactically effective β-hCG peptides. The invention provides for treatment of HIV infection by administration of a therapeutic compound of the invention.

20 The therapeutic compounds of the invention include: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, therapeutically and prophylactically effective β-hCG peptides, related derivatives and analogs of hCG, β-hCG or β-hCG peptides, and nucleic acids encoding β-hCG and β-hCG peptides, and analogs and derivatives

25 thereof. β-hCG peptides which are effective for treatment and prevention of HIV infection can be identified by *in vitro* and *in vivo* assays such as those described herein.

In a preferred embodiment, a therapeutic composition of the invention comprises a β-hCG peptide, the amino acid sequence of which consists of amino acid numbers 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-

5   65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2), particularly a β-hCG peptide which consists of amino acid numbers 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4, or 7, respectively), most preferably of a β-hCG peptide which consists of amino acid numbers 47-53 (SEQ ID NO:5) or 45-57 (SEQ ID NO:6).

10   In another preferred embodiment, a therapeutic composition of the invention comprises a fusion protein comprising two or more β-hCG sequences (either as non-contiguous or contiguous sequences), e.g. having an amino acid sequence of one β-hCG peptide linked via a peptide bond to another β-hCG peptide, in particular a protein, the amino acid sequence of which consists of amino acids

15   45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID

20   NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2), i.e., the peptides denoted 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively).

In other preferred embodiments, the therapeutic comprises a β-hCG peptide, the amino acid sequence of which consists of circularized (via a disulfide bond

25   between its amino- and carboxy-terminal cysteines) 44-57 (SEQ ID NO:26) with the valine at position 44 substituted with cysteine ((Val44Cys) 45-57 circularized) (depicted in Figure 9B), the circularized (via a disulfide bond between its amino- and carboxy- terminal cysteines) fused peptide of amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the

38

N-terminus of amino acids 45-57 (SEQ ID NO:6), or the peptide 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the branches are made up of diaminobutyric acid peptide bonded to a proline residue (depicted in Figure 9A). The amino acid sequence

5   of β-hCG is depicted in Figure 8 (SEQ ID NO:2).

In yet another embodiment, the therapeutic comprises fractions, preferably fractions of a source of hCG or β-hCG, such as commercial hCG preparations and human early pregnancy urine, of material eluting from a gel filtration column with apparent molecular weights of approximately 40 kD, 15 kD or 2-3

10   kD as determined based on in which fractions native hCG dimer (77kD) and β-hCG core protein (10 kD) elute.

The present invention further relates to therapeutic methods and compositions for treatment and prevention of wasting syndromes based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source

15   of hCG or β-hCG, and therapeutically and prophylactically effective β-hCG peptides. The invention provides for treatment of wasting syndrome by administration of a therapeutic compound (termed herein "Therapeutic") of the invention. Such Therapeutics of the invention include but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a

20   source of native hCG or native β-hCG, therapeutically and prophylactically effective β-hCG proteins (i.e., those peptides which prevent or treat wasting syndrome), related derivatives and analogs of hCG, β-hCG or β-hCG peptides, and nucleic acids encoding β-hCG and β-hCG peptides, and analogs and derivatives thereof. β-hCG peptides which are effective for treatment and

25   prevention of wasting syndromes can be identified by in vitro and in vivo assays such as those described in herein.

The present invention also relates to therapeutic methods and compositions for treatment and prevention of diseases and disorders in which increased amounts

39

of hematopoietic cells are desirable (e.g., disorders associated with reduced numbers of one or more hematopoietic cell types) by administration of hCG, β-hCG and therapeutically or prophylactically effective proteins (e.g., peptides) having a sequence of one or more portions of β-hCG (β-hCG peptides), and

5 derivatives and analogs thereof, and therapeutically or prophylactically effective fractions of a source of native hCG or native β-hCG (i.e. from a naturally occurring source and not hCG or β-hCG which has been recombinantly produced). The invention provides for treatment and prevention of hematopoietic cell deficiencies by administration either of a therapeutic

10 compound of the invention or of hematopoietic cells, the numbers of which have been increased in vitro by contact with a therapeutic compound of the invention. The invention also provides methods for expansion of hematopoietic cells in vitro by contact with a Therapeutic of the invention. The therapeutic compounds of the invention include, but are not limited to: hCG, β-hCG,

15 therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, and therapeutically and prophylactically effective β-hCG peptides, i.e., those fractions and peptides which prevent or treat HIV infection (e.g., as demonstrated in in vitro and in vivo assays described infra), and derivatives and analogs thereof, as well as nucleic acids encoding hCG, β-hCG

20 and therapeutically and prophylactically effective β-hCG peptides and derivatives and analogs thereof (e.g., for use in gene therapy).

In a preferred embodiment, a therapeutic composition of the invention comprises a β-hCG peptide, the amino acid sequence of which consists of amino acid numbers 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55,

25 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2), particularly a β-hCG peptide which consists of amino acid numbers 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4, or 7, respectively), most

preferably of a β-hCG peptide which consists of amino acids 47-53 (SEQ ID NO:5) or 45-57 (SEQ ID NO:6).

In other preferred embodiments, the therapeutic comprises a β-hCG peptide, the amino acid sequence of which consists of circularized (via a disulfide bond 5 between its amino- and carboxy- terminal cysteines) 44-57 (SEQ ID NO:26) with the valine at position 44 substituted with cysteine ((Val44Cys) 45-57 circularized) (depicted in Figure 9B), 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the branches are made up of diaminobutyric acid peptide bonded to a proline 10 residue (depicted in Figure 9A).

In another embodiment, a protein is used which contains the amino acid sequence of two or more peptides of different portions of the β-hCG sequence (either as non-contiguous or contiguous sequenced), e.g., in which the N-terminus of one portion is linked to the C-terminus of another portion by 15 peptide bond(s). In a specific embodiment, a protein is used, the amino acid sequence of which consists of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or a protein is used that has an amino 20 acid sequence of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence; i.e., the fused peptides represented as 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). In yet another embodiment a protein is used which is a cyclic, fused peptide, 25 particularly a cyclic, fused peptide having a sequence consisting of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and being

41

circularized by a disulfide bond between the terminal cysteines at positions 110 and 57.

In another embodiment, a protein is used that (a) comprises a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-
5   119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-119, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2) and (b) lacks β-hCG amino acids contiguous to said sequence. The amino acid sequence of β-hCG is depicted in Figure 8 (SEQ ID
10   NO:2).

The present invention further relates to therapeutic methods and compositions for treatment and prevention of cancers based on hCG and β-hCG preparations, therapeutically and prophylactically effective fractions of a source of hCG or β-hCG, and therapeutically and prophylactically effective β-hCG peptides. The
15   invention provides for treatment of cancer by administration of a therapeutic compound (termed herein "Therapeutic") of the invention. Such Therapeutics of the invention include but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, therapeutically and prophylactically effective β-hCG proteins (i.e., those
20   peptides which prevent or treat cancer), related derivatives and analogs of hCG, β-hCG or β-hCG peptides, and nucleic acids encoding β-hCG and β-hCG peptides, and analogs and derivatives thereof. β-hCG peptides and fractions of a source of native hCG or β-hCG which are effective for treatment and prevention of cancer can be identified by in vitro and in vivo assays such as
25   those described in herein.

In a preferred embodiment, a therapeutic composition of the invention comprises a β-hCG peptide, the amino acid sequence of which consists of amino acid numbers 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55,

42

45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2), particularly a β-hCG peptide which consists of amino acid numbers 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4, or 7, respectively), most

5   preferably of a β-hCG peptide which consists of amino acid numbers 47-53 (SEQ ID NO:5) or 45-57 (SEQ ID NO:6).   In another preferred embodiment, a therapeutic composition of the invention comprises a fusion protein comprising more than one β-hCG sequence (preferably non-contiguous sequences), e.g. having an amino acid sequence of one β-hCG peptide linked via a peptide bond

10   to another β-hCG peptide, in particular a protein, the amino acid sequence of which consists of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID

15   NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2), i.e., the peptides denoted 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively).

In other preferred embodiments, the therapeutic comprises a β-hCG peptide, the

20   amino acid sequence of which consists of circularized (via a disulfide bond between its amino- and carboxy-terminal cysteines) 44-57 (SEQ ID NO:26) with the valine at position 44 substituted with cysteine ((Val44Cys) 45-57 circularized) (depicted in Figure 9B), the circularized (via a disulfide bond between its amino- and carboxy- terminal cysteines) fused peptide of amino

25   acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6), or the peptide 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the branches are made up of diaminobutyric acid peptide

43

bonded to a proline residue (depicted in Figure 9A). The amino acid sequence of β-hCG is depicted in Figure 8 (SEQ ID NO:2).

In yet another embodiment, the therapeutic comprises fractions, preferably gel filtration fractions of a source of native hCG or native β-hCG (*i.e.* from or derived from a naturally occurring source of hCG or β-hCG and not recombinantly produced hCG or β-hCG), such as commercial hCG preparations and human pregnancy (preferably early, *i.e.* first trimester) urine, of material eluting from a SUPERDEX™ 200 (Pharmacia) gel filtration column with apparent molecular weights of approximately 40 kD, 15 kD or 2-3 kD as determined based on in which fractions native hCG dimer (77kD) and β-hCG core (10 kD) elute.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1   β-hCG PEPTIDES AND DERIVATIVES THEREOF

The invention provides isolated proteins (e.g., peptides), the amino acid sequences of which consist of one or more portions of the β-hCG sequence (β-hCG peptides), and derivatives thereof, which are effective for treatment or prevention of HIV infection and resulting disorders. In various specific embodiments, the portions of the β-hCG sequence are at least 3, 5, 10, 20, or 30 amino acids. Effectiveness of the peptides of the invention for treatment or prevention of HIV infection can be determined by any of the methods disclosed herein or by any method known in the art. In a specific embodiment, the peptides inhibit HIV infection or replication. In a preferred embodiment, the invention relates to proteins, the amino acid sequences of which consist of amino acid numbers 41-54, 45-54, 47-53 and 45-57 (SEQ ID NOS:3-6) of the β-hCG sequence depicted in Figure 8 (a portion of SEQ ID NO:2). In other embodiments, proteins, the amino acid sequences of which consist of amino

44

acid numbers 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, and 48-56 (SEQ ID NOS:7-25 and 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2) are also provided.

5   In another embodiment, the invention provides proteins, the amino acid sequences of which consist of two or more at least 5, 7 or 10 amino acid, non-naturally contiguous portions of the β-hCG sequence (Figure 8 (SEQ ID NO:2)) linked by peptide bonds between the N-terminus of one portion and the C-terminus of another portion. Specifically, proteins, the amino acid sequences

10   of which consist of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of a peptide of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-

15   terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2), i.e., the fused peptides denoted as 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). Derivatives of the foregoing fusion proteins are also provided (e.g., branched, cyclized, N- or C-terminal chemically modified, etc.). In

20   another embodiment, fusion proteins comprising two or more such portions of the β-hCG sequence are provided; such portions may or may not be contiguous to one another (i.e., an intervening sequence may be present). Molecules comprising such portions linked by hydrocarbon linkages are also provided. In another embodiment, the peptides of the invention (i) have an amino acid

25   sequence consisting of no more than 8 peptides of the β-hCG sequence (Figure 8 (SEQ ID NO:2)) and (ii) comprise amino acid numbers 47-53 (SEQ ID NO:5) of β-hCG (Figure 8 (SEQ ID NO:2)).

In another embodiment, the invention provides an isolated protein which protein (a) comprises one or more portions of the amino acid sequence of β-

45

hCG, a peptide consisting of said portion(s) being effective to inhibit HIV infection or replication; and (b) lacks β-hCG amino acids contiguous to said portion(s). In a specific embodiment, the invention provides an isolated protein (a) comprising a β-hCG amino acid sequence consisting of amino acid numbers

5 41-54, 45-54, 47-53, 45-57, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-56, 47-58, 58-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-6, 8-19, 21, 22, 24, or 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2); and (b) lacking β-hCG amino acids contiguous to said sequence.   Peptides containing the above sequences in which only

10 conservative substitutions have been made are also provided by the present invention, as but one example of peptide derivatives within the scope of the invention. Analogs of the above-mentioned proteins and peptides which have one or more amino acid substitutions forming a branched peptide (e.g., by substitution with an amino acid or amino acid analog having a free amino- or

15 carboxy-side chain that forms a peptide bond with a sequence of one or more amino acids, including but not limited to prolines) or allowing circularization of the peptide (e.g., by substitution with a cysteine, or insertion of a cysteine at the amino- or carboxy-terminus or internally), to provide a sulfhydryl group for disulfide bond formation, are also provided.

20 In specific embodiments, peptides of less than 50, or less than 25, amino acids are provided.

The invention also relates to derivatives, modifications and analogs of β-hCG peptides. In one embodiment, β-hCG peptide derivatives can be made by altering the β-hCG peptide sequence by substitutions, additions or deletions that

25 provide for therapeutically effective molecules. Thus, the β-hCG peptide derivatives include peptides containing, as a primary amino acid sequence, all or part of the particular β-hCG amino acid sequence including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a peptide which is functionally

active. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration.

Conservative substitutions for an amino acid within the sequence may be
5   selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include
10   arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such β-hCG peptide derivatives can be made either by chemical peptide synthesis or by recombinant production from nucleic acid encoding the β-hCG peptide which nucleic acid has been mutated. Any technique for mutagenesis known in the art can be used,
15   including but not limited to, chemical mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, *J. Biol. Chem* 253:6551), use of TAB® linkers (Pharmacia), etc.

In addition, β-hCG peptides and analogs and derivatives of β-hCG peptides can be chemically synthesized (see, e.g., Merrifield, 1963, *J. Amer. Chem. Soc.*
20   85:2149-2156). For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 50-60). β-hCG peptides can also be synthesized by use of a peptide synthesizer. The
25   composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 34-49). Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the β-hCG

47

peptide. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ϵ-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine,

5   norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L

10   (levorotary).

By way of example but not by way of limitation, peptides of the invention can be chemically synthesized and purified as follows: Peptides can be synthesized by employing the N-α-9-fluorenylmethyloxycarbonyl or Fmoc solid phase peptide synthesis chemistry using a Rainin Symphony Multiplex Peptide

15   Synthesizer. The standard cycle used for coupling of an amino acid to the peptide-resin growing chain generally includes: (1) washing the peptide-resin three times for 30 seconds with N,N-dimethylformamide (DMF); (2) removing the Fmoc protective group on the amino terminus by deprotection with 20% piperdine in DMF by two washes for 15 minutes each, during which process

20   mixing is effected by bubbling nitrogen through the reaction vessel for one second every 10 seconds to prevent peptide-resin settling; (3) washing the peptide-resin three times for 30 seconds with DMF; (4) coupling the amino acid to the peptide resin by addition of equal volumes of a 250 mM solution of the Fmoc derivative of the appropriate amino acid and an activator mix consisting

25   or 400 mM N-methylmorpholine and 250 mM (2-(1H-benzotriazol-1-4))-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in DMF; (5) allowing the solution to mix for 45 minutes; and (6) washing the peptide-resin three times for 30 seconds of DMF. This cycle can be repeated as necessary with the appropriate amino acids in sequence to produce the desired peptide.

30   Exceptions to this cycle program are amino acid couplings predicted to be

difficult by nature of their hydrophobicity or predicted inclusion within a helical formation during synthesis. For these situations, the above cycle can be modified by repeating step 4 a second time immediately upon completion of the first 45 minute coupling step to "double couple" the amino acid of interest.

5   Additionally, in the first coupling step in peptide synthesis, the resin can be allowed to swell for more efficient coupling by increasing the time of mixing in the initial DMF washes to three 15 minute washes rather than three 30 second washes.

After peptide synthesis, the peptide can be cleaved from the resin as follows:
10   (1) washing the peptide-resin three times for 30 seconds with DMF; (2) removing the Fmoc protective group on the amino terminus by washing two times for 15 minutes in 20% piperdine in DMF; (3) washing the peptide-resin three times for 30 seconds with DMF; and (4) mixing a cleavage cocktail consisting of 95% trifluoroacetic acid (TFA), 2.4% water, 2.4% phenol, and
15   0.2% triisopropysilane with the peptide-resin for two hours, then filtering the peptide in the cleavage cocktail away from the resin, and precipitating the peptide out of solution by addition of two volumes of ethyl ether.

To isolate the peptide, the ether-peptide solution can be allowed to sit at -20°C for 20 minutes, then centrifuged at 6,000xG for 5 minutes to pellet the peptide,
20   and the peptide can be washed three times with ethyl ether to remove residual cleavage cocktail ingredients. The final peptide product can be purified by reversed phase high pressure liquid chromatography (RP-HPLC) with the primary solvent consisting of 0.1% TFA and the eluting buffer consisting of 80% acetonitrile and 0.1% TFA. The purified peptide can then be lyophilized to a
25   powder.

In a preferred embodiment, the invention provides a peptide with branched amino acids (branched peptide), preferably a branched peptide of amino acids 45-57 (SEQ ID NO:6) with branches occurring at positions 47 and 51,

49

respectively, instead of the Gly and Ala residues normally present. Most preferably, diaminobutyric acid is substituted for the gly and ala residues at positions 47 and 51, respectively, and proline bonded to both diaminobutyric acid residues (45-57 branched) as shown in Figure 9A.

5   In other specific embodiments, branched versions of the β-hCG peptides listed hereinabove are provided, e.g., by substituting one or more amino acids within the β-hCG sequence with an amino acid or amino acid analog with a free side chain capable of forming a peptide bond with one or more amino acids (and thus capable of forming a "branch").

10   Branched peptides may be prepared by any method known in the art for covalently linking any naturally occurring or synthetic amino acid to any naturally occurring or synthetic amino acid in a peptide chain which has a side chain group able to react with the amino or carboxyl group on the amino acids so as to become covalently attached to the peptide chain. In particular, amino

15   acids with a free amino side chain group, such as, but not limited to, diaminobutyric acid, lysine, arginine, ornithine, diaminopropionic acid and citrulline, can be incorporated into a peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free amino side group, from that residue. Alternatively, amino acids with a free carboxyl side

20   chain group, such as, but not limited to, glutamic acid, aspartic acid and homocitrulline, can be incorporated into the peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free carboxyl side group, from that residue. The amino acid forming the branch can be linked to a side chain group of an amino acid in the peptide chain by any

25   type of covalent bond, including, but not limited to, peptide bonds, ester bonds and disulfide bonds. In a specific embodiment, amino acids, such as those described above, that are capable of forming a branch point, are substituted for β-hCG residues within a peptide having a β-hCG sequence.

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Branched peptides can be prepared by any method known in the art. For example, but not by way of limitation, branched peptides can be prepared as follows: (1) the amino acid to be branched from the main peptide chain can be purchased as an N-α-tert-butyloxycarbonyl (Boc) protected amino acid pentafluorophenyl (Opfp) ester and the residue within the main chain to which this branched amino acid will be attached can be an N-Fmoc-α-γ-diaminobutyric acid; (2) the coupling of the Boc protected amino acid to diaminobutyric acid can be achieved by adding 5 grams of each precursor to a flask containing 150 ml DMF, along with 2.25 ml pyridine and 50 mg dimethylaminopyridine and allowing the solution to mix for 24 hours; (3) the peptide can then be extracted from the 150 ml coupling reaction by mixing the reaction with 400 ml dichlormethane (DCM) and 200 ml 0.12 N HCl in a 1 liter separatory funnel, and allowing the phases to separate, saving the bottom aqueous layer and re-extracting the top layer two more times with 200 ml 0.12 N HCl; (4) the solution containing the peptide can be dehydrated by adding 2-5 grams magnesium sulfate, filtering out the magnesium sulfate, and evaporating the remaining solution to a volume of about 2-5 ml; (5) the dipeptide can then be precipitated by addition of ethyl acetate and then 2 volumes of hexanes and then collected by filtration and washed two times with cold hexanes; and (6) the resulting filtrate can be lyophilized to achieve a light powder form of the desired dipeptide. Branched peptides prepared by this method will have a substitution of diaminobutyric acid at the amino acid position which is branched. Branched peptides containing an amino acid or amino acid analog substitution other than diaminobutyric acid can be prepared analogously to the procedure described above, using the N-F-moc coupled form of the amino acid or amino acid analog.

In a preferred embodiment, the peptide is a cyclic peptide, preferably a cyclic peptide of β-hCG amino acids 44-57 with cysteine substituted for valine at position 44 (SEQ ID NO:26) and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 (C[V44C] 45-57) (Figure 9B), or a cyclic

51

fused peptide of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and circularized via a disulfide bond between the cysteine residues at positions 110 and 57. In another preferred embodiment, the peptide is a cyclic

5   branched peptide of β-hCG amino acids 44-57 (SEQ ID NO:26) with cysteine substituted for valine at position 44 and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 and positions 47 and 51 substituted with a diaminobutyric acid residue on which a proline is peptide bonded to its free amino sidechain.

10 Cyclization can be, for example but not by way of limitation, via a disulfide bond between two cysteine residues or via an amide linkage. For example, but not by way of limitation, disulfide bridge formation can be achieved by (1) dissolving the purified peptide at a concentration of between 0.1.-0.5 mg/ml in 0.01 M ammonium acetate, pH 7.5; (2) adding 0.01 M potassium ferricyanide

15 to the dissolved peptide dropwise until the solution appears pale yellow in color and allowing this solution to mix for 24 hours; (3) concentrating the cyclized peptide to 5-10 ml of solution, repurifying the peptide by reverse phase-high pressure liquid chromatography (RP-HPLC) and finally lyophilizing the peptide. In a specific embodiment, in which the peptide does not contain

20 two appropriately situated cysteine residues, cysteine residues can be introduced at the amino-terminus and/or carboxy-terminus and/or internally such that the peptide to be cyclized contains two cysteine residues spaced such that the residues can form a disulfide bridge.

Alternatively, a cyclic peptide can be obtained by generating an amide linkage.

25 An amide linkage can be achieved by, for example, but not limited to, the following procedure: An allyl protected amino acid, such as aspartate, glutamate, asparagine or glutamine, can be incorporated into the peptide as the first amino acid, and then the remaining amino acids coupled on. The allyl protective group can be removed by a two hour mixing of the peptide-resin with

52

a solution of tetrakistriphenylphophine palladium (0) in a solution of chloroform containing 5% acetic acid and 2.5% N-methylmorpholine. The peptide resin can be washed three times with 0.5% N,N-diisopropylethylamine (DIEA) and 0.5% sodium diethyldithiocabamate in DMF. The amino terminal Fmoc group on the peptide chain can be removed by two incubations for 15 minutes each in 20% piperdine in DMF, and washed three times with DMF for 30 seconds each. The activator mix, N-methylmorpholine and HBTU in DMF, can be brought onto the column and allowed to couple the free amino terminal end to the carboxyl group generated by removal of the allyl group to cyclize the peptide. The peptide can cleaved from the resin as described in the general description of chemical peptide synthesis above and the peptide purified by reverse phase-high pressure liquid chromatography (RP-HPLC). In a specific embodiment, in which the peptide to be cyclized does not contain an allyl protected amino acid, an allyl protected amino acid can be introduced into the sequence of the peptide, at the amino-terminus, carboxy-terminus or internally, such that the peptide can be cyclized.

β-hCG peptides can also be obtained by recombinant expression techniques. (See, e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d Ed., Cold Spring Harbor, New York, Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K., Vol. I, II). The nucleic acid sequence encoding hCG has been cloned and the sequence determined (Figure 8 (SEQ ID NOS:1 and 2) and Xia, H., 1993, *J. Molecular Endocrinology* June 10; 1993:337-343; Sherman, G.B., 1992, *J. Molecular Endocrinology*, June 6, 1992:951-959; Gieseman, L.K. (ed.), 1991, *Basic and Chemical Endocrinology*, pp. 543-567; Ward et al., 1991, in *Reproduction in Domestic Animals*, 4th ed., P.T. Coppos, ed., pp. 25-80, Academic Press, New York) and can be isolated using well-known techniques in the art, such as screening a library, chemical synthesis, or polymerase chain reaction (PCR).

To recombinantly produce a β-hCG peptide, a nucleic acid sequence encoding β-hCG or a β-hCG peptide is operatively linked to a promoter such that β-hCG or a β-hCG peptide is produced from said sequence. For example, a vector can be introduced into a cell, within which cell the vector or a portion thereof is

5   expressed, producing β-hCG or one or more portions thereof. In a preferred embodiment, the nucleic acid is DNA if the source of RNA polymerase is DNA-directed RNA polymerase, but the nucleic acid may also be RNA if the source of polymerase is RNA-directed RNA polymerase or if reverse transcriptase is present in the cell or provided to produce DNA from the RNA. Such a vector

10   can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in bacterial or mammalian cells. Expression of the sequence encoding β-hCG

15   or the β-hCG peptide can be by any promoter known in the art to act in bacterial or mammalian cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell*

20   22:787-797), the HSV-1 (herpes simplex virus-1) thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42), etc., as well as the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase

25   I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid

30   cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse

54

mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver

5 (Krumlauf et al., 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer et al., 1987, *Science* 235:53-58), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, *Genes and Devel.* 1:161-171), beta-globin gene control region which is active in erythroid cells (Mogram et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46, 89-94), myelin basic protein gene

10 control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286), and gonadotropin releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378). The promoter element which is

15 operatively linked to the nucleic acid encoding β-hCG or a β-hCG peptide can also be a bacteriophage promoter with the source of the bacteriophage RNA polymerase expressed from a gene for the RNA polymerase on a separate plasmid, e.g., under the control of an inducible promoter, for example, the nucleic acid encoding β-hCG or β-hCG peptide operatively linked to the T7 RNA

20 polymerase promoter with a separate plasmid encoding the T7 RNA polymerase.

In a less preferred embodiment, peptides can be obtained by proteolysis of hCG or β-hCG followed by purification using standard techniques such as chromatography (e.g., HPLC), electrophoresis, etc.

Also included within the scope of the invention are β-hCG peptide derivatives

25 which are differentially modified during or after synthesis, e.g., by benzylation, glycosylation, acetylation, phosphorylation, amidation, pegylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. In specific

embodiments, the peptides are acetylated at the N-terminus and/or amidated at the C-terminus.

Any of numerous chemical modifications may be carried out by known techniques, including but not limited to acetylation, formylation, oxidation,
5   reduction; metabolic synthesis in the presence of tunicamycin; etc.

In another embodiment, the β-hCG or β-hCG peptide derivative is a chimeric, or fusion, protein comprising β-hCG or a functional β-hCG peptide (or two or more portions of β-hCG joined by peptide bond(s)) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different
10   protein. In a specific embodiment, the derivative is a fusion protein comprising the β-hCG sequence, or portions thereof, joined at its amino or carboxy-terminus to an amino acid sequence, or portions thereof, of a chemokine which is therapeutically useful in the treatment of AIDS, for example, the chemokines MIP-1α, MIP-1β or Rantes (for amino acid sequences of these chemokines see
15   Shall, 1991, *Cytokine* <u>3</u>:165-183). In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a β-hCG-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences
20   to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer.

5.2 THERAPEUTIC USES

5.2.1 HIV

The invention provides for treatment or prevention of diseases and disorders associated with HIV infection by administration of a therapeutic compound
5   (termed herein "Therapeutic"). Such "Therapeutics" include, but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG, and therapeutically and prophylactically effective β-hCG peptides, *i.e.*, those fractions and peptides which prevent or treat HIV infection (*e.g.*, as demonstrated in *in vitro* and *in vivo* assays
10   described *infra*), and derivatives and analogs thereof, as well as nucleic acids encoding hCG, β-hCG and therapeutically and prophylactically effective β-hCG peptides and derivatives and analogs thereof (*e.g.*, for use in gene therapy). Examples of Therapeutics are those proteins described herein and nucleic acids encoding such proteins and frations of native hCG and native β-hCG described
15   below.

A preferred embodiment of the invention relates to methods of using a Therapeutic for treatment or prevention of HIV infection, preferably HIV-1 infection, in a human subject. In a specific embodiment, the Therapeutic is used for the treatment or prevention of HIV infection in a human subject that
20   does not suffer from a cancer which secretes hCG or hCG fragments. In another specific embodiment, the Therapeutic is used for the treatment or prevention of HIV infection in a human subject who does not suffer from Kaposi's sarcoma (KS). In the treatment of HIV infection, the Therapeutic of the invention can be used to prevent progression of HIV infection to ARC or to AIDS in a human
25   patient, or to treat a human patient with ARC or AIDS.

In a preferred aspect of the invention, proteins, preferably β-hCG peptides and derivatives are used to treat HIV infection. In particular, proteins, or nucleic acids encoding the proteins, containing an amino acid sequence of one or more

portions of β-hCG, preferably containing a sequence from amino acids 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2), and

5   preferably containing a sequence from amino acids 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4 or 7, respectively) of Figure 8 (a portion of SEQ ID NO:2), and most preferably containing a sequence from amino acids 47-53 or 45-57 of Figure 8 (a portion of SEQ ID NO:2), or circular [C44V]45-57 peptide (SEQ ID NO:26), or branched 45-57 (SEQ ID NO:6) peptide, or branched circular

10   [V44C]45-57 peptide are used to treat HIV infection. In another embodiment, a protein is used which contains the amino acid sequence of two or more peptides of different, non-naturally contiguous portions of the β-hCG sequence (Figure 8 (SEQ ID NO:2)) in which the N-terminus of one portion is linked to the C-terminus of another portion by peptide bond(s). In a specific embodiment, a

15   protein is used, the amino acid sequence of which consists of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or a protein is used that has an amino acid sequence of amino acids 47-57 (SEQ ID NO:28)

20   linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2); i.e., the fused peptides represented as 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32, respectively). In yet another embodiment a protein is used which is a cyclic, fused peptide,

25   particularly a cyclic, fused peptide having a sequence consisting of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and being circularized by a disulfide bond between the terminal cysteines at positions 110 and 57. In another embodiment, a protein is used that (a) comprises a β-hCG

30   amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55,

58

45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-119, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2) and (b) lacks β-hCG amino acids contiguous to said sequence. In yet another embodiment, a purified derivative

5   of a protein is used to treat or prevent HIV infection, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as

10   depicted in Figure 8 (a portion of SEQ ID NO:2). Other β-hCG peptides, and nucleic acids encoding the peptides, and modifications and derivatives thereof, may have utility in the therapeutic methods of the invention.

In yet another embodiment, a fraction, particularly a size fraction, of a source of native hCG or native β-hCG (i.e. naturally occurring, not recombinantly

15   produced, hCG or β-hCG) active in inhibiting HIV infection and replication, particularly a size fraction of approximately 40 kD, 15 kD or 2-3 kD, is used to treat or prevent HIV infection. The utility of β-hCG peptides and fractions of native hCG and native β-hCG sources may be determined by the in vitro and in vivo assays described herein or by any other method known in the art.

20   Additionally, the present inventors have found that different preparations of native hCG and native β-hCG have variable effects on HIV infection both in vitro and in vivo. Specifically, the inventors found that among the commercial preparations of (non-recombinant) hCG they investigated, hCG from Fujisawa was the most effective, hCG APL™ (Wyeth-Ayerst) the next most effective, and

25   PREGNYL™ (Organon) the next most effective in inhibiting HIV infection and replication. A highly purified hCG preparation and recombinant β-hCG were found not to be active in inhibiting HIV infection in vitro. In fact, the present inventors have shown that specific size fractions of an active hCG preparation

59

(APL™; Wyeth Ayerst) have anti-HIV activity in vitro and anti-KS activity both in vitro and in vivo, as described herein.

These active fractions were eluted from the gel filtration sizing matrix as or close to (i.e., within 5 fractions (where the fractions are 4 ml fractions using a

5   SUPERDEX™ 200 column which is 26 mm² by 60 mm)) the fractions containing or that would contain material that is approximately 40 kD (±8 kD), 15 kD (±3 kD) and 2-3 kD (±2 kD) molecular weight.   One skilled in the art would understand that these fractions could be subjected to further size fractionation to further isolate the component of these fractions having the anti-HIV and/or

10   anti-KS activity.   Additionally, other methods of fractionation, such as ion-exchange chromatography, affinity chromatography, etc., are well known in the art; those skilled in the art would be able to use any available fractionation techniques to obtain the active fractions from the active hCG preparations and human early (first trimester) pregnancy urine. hCG preparations and fractions

15   of hCG preparations can be screened for efficacy in treating or preventing HIV infection, cancer, wasting syndrome, and/or hematopoietic deficiencies by the assays described herein or by any method known in the art. In a specific embodiment, the invention provides a first composition comprising one or more first components of a second composition comprising native hCG or native β-

20   hCG, said first components being separated from other components of the hCG or β-hCG sample, said first components being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, and said second composition being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, and said native hCG

25   or native β-hCG not being purified to homogeneity in said second composition. In particular the invention provides a composition comprising components which have been separated from other components of the native hCG or native β-hCG sample by sizing column chromatography, preferably where the components elute from a gel filtration, preferably a SUPERDEX™ 200, sizing

30   column with an apparent approximate molecular weight of 40 kD, 14 kD or 2-3

kD as determined relative to the elution of a native hCG heterodimer, having a molecular weight of 77 kD, and a β-hCG core protein (β-hCG amino acids 6-40 linked via a disulfide bond to β-hCG amino acids 55-92, as depicted in Figure 8 (SEQ ID NO:2)), having a molecular weight of 10 kD. In a specific

5   embodiment, the therapeutic method of the invention is carried out as monotherapy, *i.e.*, as the only agent provided for treatment or prevention of HIV. In another embodiment, the Therapeutic is administered in combination with one or more anti-viral compounds, for example, protease inhibitors (*e.g.*, saquinavir) and/or reverse transcriptase inhibitors (*e.g.*, azidothymidine (AZT),

10   lamioridine (3TC), dideoxyinosine (ddI), dideoxycytidine (ddC)). The Therapeutic may also be administered in conjunction with chemotherapy (*e.g.*, treatment with adriamycin, bleomycin, vincristine, vinblastine, doxorubicin and/or Taxol) or other therapies known in the art.

In another embodiment, HIV infection is treated or prevented by administration

15   of a Therapeutic of the invention in combination with one or more chemokines. In particular, the Therapeutic is administered with one or more C-C type chemokines, especially one or more from the group RANTES, MIP-1α and MIP-1β.

The invention provides for treatment or prevention of wasting syndrome by

20   administration of a therapeutic compound (termed herein "Therapeutic"). Such "Therapeutics" include, but are not limited to: hCG, β-hCG, therapeutically and prophylactically effective fractions of a source of native hCG or native β-hCG (*i.e.* naturally occurring hCG or β-hCG and not recombinantly produced hCG or β-hCG), and therapeutically and prophylactically effective β-hCG peptides, *i.e.*,

25   those fractions and peptides which prevent or treat wasting syndrome (*e.g.*, as demonstrated in *in vitro* and *in vivo* assays described *infra*), and derivatives and analogs thereof, as well as nucleic acids encoding hCG, β-hCG and

61

therapeutically and prophylactically effective β-hCG peptides and derivatives and analogs thereof (e.g., for use in gene therapy).

The methods of the invention can be used for treatment or prevention of any disease or disorder characterized by a loss of body cell mass. Particular
5 conditions that can be treated by methods of the invention include, but are not limited to, wasting associated with viral, such as HIV, bacterial or other types of infections, and sepsis; cachexia associated with cancer, chemotherapy, and radiation therapy; wasting associated with chronic cardiovascular disease; wasting caused by exposure to toxic substances; wasting associated with
10 diarrhea and other gastrointestinal disorders.

In a preferred embodiment, a Therapeutic of the invention is administered to treat or prevent a wasting syndrome associated with HIV infection. In another preferred embodiment, a Therapeutic of the invention is administered to treat or prevent a wasting syndrome associated with cancer.

15 **5.2.2 HEMATOPOIESIS**

The invention also provides for treatment or prevention of diseases and disorders in which increased numbers of one or more hematopoietic cell types are desirable (e.g., diseases or disorders associated with one or more hematopoietic cell deficiencies) by administration of a therapeutic compound
20 (termed herein "Therapeutic") of the invention or by administration of hematopoietic cells, the production of which has been induced in vitro by contacting the cells with a Therapeutic of the invention. Such "Therapeutics" include but are not limited to: hCG, β-hCG and derivatives thereof, and therapeutically or prophylactically effective fractions of a source of native hCG
25 or native β-hCG and therapeutically or prophylactically effective β-hCG peptides, i.e., those fraction and peptides which prevent or treat hematopoietic deficiencies (e.g., as demonstrated in in vitro and in vivo assays described infra)

62

as well as modifications, derivatives and analogs thereof and nucleic acids encoding hCG, β-hCG and therapeutically and prophylactically effective β-hCG peptides, and derivatives and analogs thereof. In one embodiment, the Therapeutic of the invention is a protein containing an amino acid sequence of

5   a therapeutically and prophylactically effective portion or portions of β-hCG.

In a preferred embodiment, the Therapeutic of the invention is a protein having a sequence of amino acid numbers 41-54, 45-54, 47-53 or 45-57 (SEQ ID NOS:3-6, respectively) of the β-hCG sequence depicted in Figure 8 (a portion

10   of SEQ ID NO:2). In other embodiments, the Therapeutic of the invention is a protein having a sequence of amino acid numbers 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of the β-hCG sequence of Figure 8 (a portion of SEQ ID NO:2).

15   Additionally, the present inventors have found that different preparations of hCG and β-hCG have variable effects on hematopoietic cell proliferation *in vitro* and *in vivo*. Specifically, the inventors found that among the (non-recombinant) commercial preparations they investigated, hCG from Fujisawa was the most effective, hCG from APL (Wyeth-Ayerst) the next most effective,

20   and pregnyl (Organon) the next most effective. hCG preparations and fractions of hCG and β-hCG preparations can be screened for utility in inducing hematopoiesis *in vitro* or *in vivo* by the methods described *infra* herein or any method known in the art.

In one embodiment of the invention, the Therapeutic is administered directly

25   to a patient suffering from a disease or disorder amenable to treatment by increasing production of one or more hematopoietic cell types (*e.g.*, a disease or disorder associated with a hematopoietic cell deficiency). In another embodiment of the invention, hematopoietic cells, preferably stem and/or progenitor cells, are obtained, contacted with a Therapeutic of the invention *in*

63

vitro to induce proliferation of the cells, and then administered to a subject suffering from a disease or disorder associated with a hematopoietic cell deficiency. Preferably, autologous hematopoietic cells (obtained from the subject or its identical twin) are reintroduced into the subject after *in vitro*

5 expansion. In this embodiment, gene therapy methods can be performed by introducing a nucleic acid of interest, e.g., containing a gene which provides a function desired in a subject, into the hematopoietic cells, before or after expansion of the cells by contact with a Therapeutic. Hematopoietic cell subpopulations can be isolated for use, before or after expansion *in vitro*. For

10 example, blood cells can be isolated and expanded, and optionally also differentiated, *in vitro*, followed by introduction of all or a portion of the cells (*e.g*, purified platelets, red blood cells, lymphocytes, etc.) into a patient.

In general, disorders that can be treated by methods of the invention include, but are not limited to, five broad categories. First are diseases resulting from

15 a failure or dysfunction of normal blood cell production and maturation (*i.e.*, aplastic anemia, cytopenias and hypoproliferative stem cell disorders). The second group are neoplastic, malignant diseases in the hematopoietic organs (*e.g.*, leukemia and lymphomas). The third group of disorders comprises those of patients with a broad spectrum of malignant solid tumors of non-

20 hematopoietic origin. Induction of hematopoietic cell proliferation or administration of replacement hematopoietic cells in these patients serves as a bone marrow rescue procedure, which is provided to a patient following otherwise lethal chemotherapy or irradiation of the malignant tumor. The fourth group of diseases consists of autoimmune conditions, where the hematopoietic

25 cells serve as a source of replacement of an abnormal immune system. The fifth group of diseases comprises a number of genetic disorders which can be corrected by infusion of hematopoietic stem cells, preferably syngeneic, which prior to transplantation have undergone gene therapy. Particular diseases and disorders which can be treated by induction of hematopoietic cell production

64

in vivo or by administration of hematopoietic cells expanded in vitro include but are not limited to those listed in Table 1, and described infra.

TABLE 1

DISEASES OR DISORDERS WHICH CAN BE TREATED

BY INCREASING PRODUCTION OF HEMATOPOIETIC CELLS

I. Diseases resulting from a failure or dysfunction of normal blood cell production and maturation
hyperproliferative stem cell disorders
aplastic anemia
pancytopenia
agranulocytosis
thrombocytopenia
red cell aplasia
Blackfan-Diamond syndrome
due to drugs, radiation, or infection
idiopathic

II. Hematopoietic malignancies
acute lymphoblastic (lymphocytic) leukemia
chronic lymphocytic leukemia
acute myelogenous leukemia
chronic myelogenous leukemia
acute malignant myelosclerosis
multiple myeloma
polycythemia vera
agnogenic myelometaplasia
Waldenstrom's macroglobulinemia
Hodgkin's lymphoma
non-Hodgkin's lymphoma

III. Immunosuppression in patients with malignant, solid tumors
malignant melanoma
carcinoma of the stomach
ovarian carcinoma
breast carcinoma
small cell lung carcinoma
retinoblastoma
testicular carcinoma
glioblastoma

65

rhabdomyosarcoma
neuroblastoma
Ewing's sarcoma
Lymphoma

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IV.   Autoimmune diseases
rheumatoid arthritis
diabetes type I
chronic hepatitis
10   multiple sclerosis
systemic lupus erythematosus

V.   Genetic (congenital) disorders
anemias
15   familial aplastic
Fanconi's syndrome
Bloom's syndrome
pure red cell aplasia (PRCA)
dyskeratosis congenita
20   Blackfan-Diamond syndrome
congenital dyserythropoietic syndromes I-IV
Chwachmann-Diamond syndrome
dihydrofolate reductase deficiencies
formamino transferase deficiency
25   Lesch-Nyhan syndrome
congenital spherocytosis
congenital elliptocytosis
congenital stomatocytosis
congenital Rh null disease
30   paroxysmal nocturnal hemoglobinuria
G6PD (glucose-6-phosphate dehydrogenase)
variants 1,2,3
pyruvate kinase deficiency
congenital erythropoietin sensitivity
35   deficiency
sickle cell disease and trait
thalassemia alpha, beta, gamma
met-hemoglobinemia
congenital disorders of immunity
40   severe combined immunodeficiency disease
(SCID)
bare lymphocyte syndrome
ionophore-responsive combined
immunodeficiency
45   combined immunodeficiency
with a capping abnormality
nucleoside phosphorylase deficiency

66

granulocyte actin deficiency
infantile agranulocytosis
Gaucher's disease
adenosine deaminase deficiency
5   Kostmann's syndrome
reticular dysgenesis
congenital leukocyte dysfunction syndromes

VI.   Others
10   osteopetrosis
myelosclerosis
acquired hemolytic anemias
acquired immunodeficiencies
infectious disorders causing primary or
15   secondary immunodeficiencies
bacterial infections (e.g., Brucellosis,
Listeriosis, tuberculosis, leprosy)
parasitic infections (e.g., malaria,
Leishmaniasis)
20   fungal infections
disorders involving disproportions in
lymphoid cell sets and impaired
immune functions due to aging
phagocyte disorders
25   Kostmann's agranulocytosis
chronic granulomatous disease
Chediak-Higachi syndrome
neutrophil actin deficiency
neutrophil membrane GP-180 deficiency
30   metabolic storage diseases
mucopolysaccharidoses
mucolipidoses
miscellaneous disorders involving
immune mechanisms
35   Wiskott-Aldrich Syndrome
alpha 1-antitrypsin deficiency

5.2.3 TREATMENT OF MALIGNANCIES

Malignancies and related disorders that can be treated or prevented by
40   administration of a Therapeutic of the invention include, but are not limited to,

67

those disorders listed in Table 2 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippencott Co., Philadelphia):

TABLE 2
MALIGNANCIES AND RELATED DISORDERS

Leukemia
    Acute:
        acute lymphocytic leukemia
        acute myelocytic leukemia
            myeloblastic
            promyelocytic
            myelomonocytic
            monocytic
            erythroleukemia
    Chronic:
        chronic myelocytic (granulocytic) leukemia
        chronic lymphocytic leukemia

Polycythemia vera
Lymphoma
Hodgkin's disease
non-Hodgkin's disease
Multiple myeloma
Waldenström's macroglobulinemia
Heavy chain disease
Solid tumors
    Sarcomas and Carcinomas:
        fibrosarcoma
        myxosarcoma
        liposarcoma
        chondrosarcoma
        osteogenic sarcoma
        chordoma
        angiosarcoma
        endotheliosarcoma
        lymphangiosarcoma
        Kaposi's sarcoma
        lymphangioendotheliosarcoma
        synovioma
        mesothelioma
        Ewing's tumor
        leiomyosarcoma

68

rhabdomyosarcoma
colon carcinoma
pancreatic cancer
breast cancer
5   ovarian cancer
prostate cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
10   sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
15   medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
20   choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
25   uterine cancer
testicular tumor
lung carcinoma
small cell lung carcinoma
bladder carcinoma
30   epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
35   ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
40   meningioma
melanoma
neuroblastoma
retinoblastoma
Virally induced cancers

In specific embodiments, a Therapeutic of the invention is used to treat a neoplasm such as a gestational trophoblastic tumor, or testicular germ cell tumor, or cancer of the bladder, pancreas, cervix, lung, liver, ovary, colon or

5   stomach, or adenocarcinoma or a virally induced cancer. In a preferred embodiment, a Therapeutic of the invention is used to treat neuroblastoma or carcinoma of the ovary or stomach. In a more preferred embodiment, a Therapeutic of the invention is used to treat Kaposi's sarcoma or carcinoma of the breast, lung, prostate, or kidney (renal).

10   In one aspect of the invention, the Therapeutic is administered in conjunction with other cancer therapy, such as chemotherapy (e.g., treatment with adriamycin, bleomycin, vincristine, vinblastine, doxorubicin and/or Taxol).

The efficacy of a Therapeutic against a particular cancer can be determined by any method known in the art, for example but not limited to, those methods

15   described herein.

The Therapeutics of the invention can also be administered to treat premalignant conditions and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 2. Such prophylactic or therapeutic use is indicated in conditions known or suspected

20   of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79). Hyperplasia is a form of controlled cell

25   proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of

70

controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is

5 found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found

10 in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can

15 indicate the desirability of prophylactic/ therapeutic administration of a Therapeutic of the invention. Such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, etc.

20 In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (e.g., the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or

25 Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (e.g., familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis,

71

medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-

5   Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113, etc.)

In another specific embodiment, a Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, lung, pancreatic, or

10   uterine cancer, or melanoma or sarcoma.

Hyperproliferative malignant stem cell disorders as well as non-hematopoietic malignancies can be treated with chemotherapy or radiation therapy along with rescue of hematopoietic cells by direct administration of a Therapeutic of the invention or by administration of hematopoietic cells induced to proliferate by

15   contacting the cells with a Therapeutic of the invention. The conditions that can be treated according to the invention include, but are not limited to, the leukemias listed in Table 2 and the solid tumors listed in Table 2.

These malignancies are currently treated by, *inter alia*, chemotherapy and, when feasible, allogeneic bone marrow transplantation. However, allogeneic HLA

20   identical sibling bone marrow is available only to less than one-third of patients, and this treatment is associated with transplantation-related complications such as immunodeficiency and graft versus host disease. Induction of hematopoietic cell proliferation *in vivo* or provision of autologous hematopoietic stem and progenitor cells expanded by administration of a Therapeutic

25   *in vitro* permits hematopoietic reconstitution of patients lacking suitable allogeneic donors and eliminates the risks of graft versus host disease arising from allogeneic marrow transplantation. Thus, in a specific embodiment, a Therapeutic is used to induce proliferation in hematopoietic cells which are

then administered to a patient who has undergone chemotherapy or radiation therapy for treatment of cancer or an immunological disorder. In another embodiment, a Therapeutic is directly administered to a patient who has undergone chemotherapy or radiation therapy for treatment of cancer or an

5   immunological disorder.

5.2.4 AUTOIMMUNE DISORDERS

Many chronic inflammatory and degenerative diseases are characterized by a continuous immune reaction against the body's own tissues. Such autoimmune disorders include but are not limited to rheumatoid arthritis and other inflam-

10   matory osteopathies, diabetes type I, chronic hepatitis, multiple sclerosis, and systemic lupus erythematosus. Autoimmune disorders are often treated by lymphoid irradiation. Administration of a Therapeutic of the invention or of cells produced by exposure to a Therapeutic *in vitro* can be valuable to repopulate the hematopoietic system after radiotherapy.

15   Anti-inflammatory drugs such as steroids retard the inflammatory cells which are activated by autoreactive T cells, but do not prevent T cells which recognize self-proteins from activating new inflammatory cells. A more direct approach to treating autoimmune diseases depends on eradication of T cells by irradiation of the lymphoid tissues, and relying on stem cells from the unirradiated bone

20   marrow to repopulate the patient's hematopoietic system. The rationale is that the formation of new populations of mature T cells from bone marrow stem cells may result in absence of T cells that have reactivity to self-specific antigens. This procedure, called total lymphoid irradiation (TLI), has been used to treat intractable rheumatoid arthritis (Strober, S., et al., 1985, *Annals of Internal*

25   *Medicine* <u>102</u>:441-449, 450-458). These clinical trials showed that in the majority of otherwise intractable cases, joint disease was significantly alleviated for at least 2-3 years. However, the major drawback to such treatment is failure of stem cells in the bone marrow of these elderly patients to efficiently

repopulate the hematopoietic system, resulting in infections and bleeding disorders. Analogous studies have been made of the effects of TLI as an alternative to cytotoxic drugs for treatment of SLE (Strober, S., et al., 1985, *Ann. Internal Med.* 102:450). Studies of the use of TLI to treat intractable SLE have also
5 shown that this treatment alleviates disease activity, but is severely limited by failure of bone marrow stem cells to rapidly and efficiently repopulate the hematopoietic system after irradiation.

Thus, a Therapeutic of the invention can be administered to promote proliferation of the remaining hematopoietic cells to increase the success of TLI
10 therapy. Additionally, hematopoietic stem and progenitor cells can be isolated from the patient before treatment, induced to proliferate *in vitro* and then introduced into the patient after TLI treatment to repopulate the hematopoietic system.

5.2.5 DISEASES RESULTING FROM A FAILURE OR DYSFUNCTION OF NORMAL BLOOD CELL PRODUCTION AND MATURATION
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In a preferred aspect, a Therapeutic of the invention is used to treat a disease resulting from a failure or dysfunction of normal blood cell production and maturation, such as an aplastic anemia, a cytopenia or a hypoproliferative stem cell disorder. These disorders entail failure of stem cells in bone marrow to
20 provide normal numbers of functional blood cells. The aplastic anemias result from the failure of stem cells to give rise to the intermediate and mature forms of red cells, white cells, and platelets. While red cell production is usually most seriously affected, a marked decrease in production of other mature blood cell elements is also seen as some anemias specifically affect production of
25 white cells and/or platelets. The large majority of these anemias are acquired during adult life, and do not have any apparent genetic predisposition. About half of these acquired anemias arise in the absence of any obvious causative factor such as exposure to poisons, drugs or disease processes that impair stem cell function; these are termed idiopathic aplastic anemias. The remaining

74

cases are associated with exposure to an extremely diverse array of chemicals and drugs and also occur as the consequence of viral infections, such as HIV infection, and after pregnancy. Other specific types of aplastic anemia are termed agranulocytosis or thrombocytopenia to indicate that the major

5   deficiency lies in particular white cells or in platelet production, respectively. These non red blood cell deficiencies are also often associated with HIV infection. Also significantly associated with HIV infection is a severe platelet deficiency, Idiopathic Thrombocytopenic Purpura (ITP). Additionally, agranulocytosis may be associated with autoimmune syndromes such as systemic

10   lupus erythematosus (SLE) or with other infections, such as neonatal rubella.

In addition, immune deficiencies which are the primary or secondary result of infection by pathogenic microorganisms can be treated by administration of a Therapeutic of the invention. For example, immune deficiencies caused by microorganisms which are intracellular pathogens of hematopoietic cells, can

15   be treated by the provision of new hematopoietic cells. These new hematopoietic cells can be generated by contacting hematopoietic stem and/or progenitor cells *in vitro* with a Therapeutic of the invention to cause proliferation of the cells. Microorganisms causing immune deficiencies which may be treated according to this embodiment of the invention include but are

20   not limited to gram-negative bacilli such as *Brucella* or *Listeria*, the mycobacterium which are the etiological agents of tuberculosis or of Hansen's disease (leprosy), parasites such as *Plasmodium* (the etiological agents of malaria) or *Leishmania*, and fungi (such as those that cause pneumonia and other lethal infections secondary to immunodeficiencies) (for a discussion of

25   many of these disorders, see Harrison's Principles of Internal Medicine, 1970, 6th Edition, Wintrobe, M.M., et al., eds., McGraw-Hill, New York, pp. 798-1044).

In a preferred embodiment of the invention, a Therapeutic of the invention is administered for the treatment of a cytopenia associated with HIV infection.

The hematopoietic deficiencies associated with HIV infection include reduction in CD4$^+$ T cells and other lymphocytes, red blood cells, platelets, specifically ITP, and neutrophils. Such a disorder is treated by contacting hematopoietic stem and/or progenitor cells *in vitro* with a Therapeutic of the invention and
5   then infusing the resulting hematopoietic cells into the subject in need of treatment. In a another preferred embodiment, the disorder is treated by direct administration of a Therapeutic of the invention to the subject in need of treatment. Assays for determining the efficacy of particular Therapeutics for treatment of hematopoietic deficiencies associated with HIV infection are
10   detailed herein.

5.3   SOURCES OF hCG AND β-hCG

Native preparations (*i.e.* derived from naturally occurring sources and not recombinantly produced) of hCG and β-hCG can be obtained from a variety of sources. Both hCG and β-hCG are commercially available (*e.g.*, Sigma Chemical
15   Company) and hCG is commercially available in a form suitable for therapeutic use in humans (*e.g.*, from Fujisawa, Wyeth-Ayerst Laboratories (APL™), Organon, Inc. (PREGNYL™) and Serono Laboratories, Inc. (PROFASI™)). hCG is also present at particularly high concentrations in the urine of women in the first trimester of pregnancy ("human early pregnancy urine"). Other sources
20   include, but are not limited to, urine from women in the second and third trimesters of pregnancy, urine from patients with proteinuria, urine from patients having hCG secreting tumors or other cancer patients, and from pituitary glands.

Since the inventors have discovered that different sources of hCG have variable
25   effects on HIV infection and cancer cell growth *in vitro* and *in vivo,* one aspect of the invention relates to assaying preparations of hCG for efficacy in treatment or prevention of HIV infection. The therapeutic effectiveness of hCG preparations and fractions can be tested by the *in vitro* or *in vivo* assays

described herein or by any method known in the art. It is preferable to test the hCG preparation or fraction in an *in vitro* assay, *e.g.*, for HIV replication or transcription from the HIV-1 LTR or *in vivo* in an animal model, such as HIV transgenic mice or SIV infected monkeys, before assaying the preparation in

5   humans.

In a specific embodiment, a preparation comprising hCG is used that contains not only the hCG heterodimer but also peptide fragments thereof, *e.g.*, β chain peptides.

hCG and β-hCG can also be purified, or preferably partially purified, from any

10   source known to contain hCG or β-hCG, *e.g.*, urine from pregnant women, using conventional techniques well-known in the art, such as affinity chromatography. For example, antibodies prepared against hCG or β-hCG can be used to prepare an affinity chromatography column which can be used to purify the proteins by well-known techniques (*see, e.g.*, Hudson & May, 1986, *Practical Immunology*,

15   Blackwell Scientific Publications, Oxford, United Kingdom).

The β-hCG-related proteins are preferably prepared by any chemical or enzymatic synthesis method known in the art, as described *supra* herein.

5.3.1 FRACTIONATION OF SOURCES OF hCG

The present inventors have found that the component(s) of a source of hCG

20   having therapeutic activity can be further isolated by fractionation of the source of hCG. The inventors have fractionated the active portions of the commercial hCG preparation APL™ (Wyeth-Ayerst) and human early pregnancy urine as described herein. Other sources of hCG include, but are not limited to, urine from women in the second and third trimester of pregnancy, urine from

25   proteinuria patients (both pregnant women with preeclampsia and patients with nephrotic syndromes), urine from patients with hCG secreting tumors, and

77

pituitary glands. However, those skilled in the art will appreciate that any source of hCG or β-hCG having anti-HIV activity and/or anti-KS activity and/or a pro-hematopoietic effect can be fractionated to further isolate the active components. The source of hCG or β-hCG can be fractionated using any

5   technique available in the art for the separation and isolation of molecules, for example but not limited to, sizing chromatography, ion-exchange chromatography, affinity chromatography, etc.

Additionally, the present inventors have found that different preparations of hCG and β-hCG have variable effects on HIV infection, KS cell growth and

10   hematopoiesis both *in vitro* and *in vivo*. Specifically, the inventors found that among the commercial preparations of (non-recombinant) hCG they investigated, hCG from Fujisawa was the most effective, hCG APL™ (Wyeth-Ayerst) the next most effective, and PREGNYL™ (Organon) the next most effective in inhibiting HIV infection and replication. A highly purified hCG preparation and

15   recombinant β-hCG were found not to be active in inhibiting HIV infection, KS cell growth and promoting hematopoiesis *in vitro*. In fact, the present inventors have shown that specific size fractions of an active hCG preparation (APL™; Wyeth Ayerst) and human early (*i.e.* first trimester) pregnancy urine have anti-HIV and pro-hematopoieitic activities in vitro and anti-KS activity both in vitro

20   and in vivo, as described *infra* herein.

These active fractions were eluted from the sizing matrix as or close to (i.e., within 5 fractions (where the fractions are 4 ml fractions using a SUPERDEX™ 200 (Pharmacia) column which is 26 mm^2 by 60 mm)) the fractions containing or that would contain material that is approximately 40 kD (±8 kD), 15 kD (±3

25   kD) and 2-3 kD (±2 kD) molecular weight. One skilled in the art would understand that these fractions could be subjected to further size fractionation to further isolate the component of these fractions having the anti-HIV and/or anti-KS activity. Additionally, other methods of fractionation, such as ion-exchange chromatography, affinity chromatography are well known in the art;

those skilled in the art would be able to use any available fractionation techniques to obtain the active fractions from the active hCG preparations and human early (first trimester) pregnancy urine (or any other preparation containing hCG or β-hCG).

5   In a specific embodiment, the invention provides a first composition comprising one or more first components of a second composition comprising native hCG or native β-hCG, said first components being separated from other components of the hCG or β-hCG sample, said first components being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic
10   activity, and said second composition being active to inhibit HIV infection or replication or Kaposi's sarcoma or having a pro-hematopoietic activity, and said native hCG or native β-hCG not being purified to homogeneity in said second composition.

In particular the invention provides a composition comprising components
15   which have been separated from other components of the native hCG or native β-hCG sample by sizing column chromatography, preferably where the components elute from a gel filtration, preferably a SUPERDEX™ 200, sizing column with an apparent approximate molecular weight of 40 kD, 14 kD or 2-3 kD as determined relative to the elution of a hCG heterodimer, having a
20   molecular weight of 77 kD, and a β-hCG core protein (β-hCG amino acids 6-40 linked via a disulfide bond to β-hCG amino acids 55-92, as depicted in Figure 8 (SEQ ID NO:2)), having a molecular weight of 10 kD. hCG preparations and fractions of hCG preparations can be screened for efficacy in inducing proliferation of hematopoietic cells by the assays described herein or by any
25   method known in the art.

Thus, in a preferred embodiment, a fraction, particularly a size fraction, of a source of native hCG or native β-hCG active in promoting hematopoiesis,

79

particularly a size fraction of approximately 40 kD, 15 kD or 2-3 kD, is used to treat or prevent a hematopoietic deficiency.

The inventors have fractionated the active portions of the commercial hCG preparation APL™ (Wyeth-Ayerst) and human early (*i.e.* first trimester)
5   pregnancy urine as described herein. Other sources of hCG include, but are not limited to, urine from women in the second and third trimester of pregnancy, urine from proteinuria patients (both pregnant women with preeclampsia and patients with nephrotic syndromes), urine from patients with hCG secreting tumors, and pituitary glands. However, those skilled in the art will appreciate
10   that any source of hCG or β-hCG having anti-HIV activity and/or anti-KS activity and/or a pro-hematopoietic effect can be fractionated to further isolate the active components. The source of hCG or β-hCG can be fractionated using any technique available in the art for the separation and isolation of molecules, for example but not limited to, sizing chromatography, ion-exchange
15   chromatography, affinity chromatography, etc.

Briefly, by way of example but not by way of limitation, urine can be prepared for fractionation as follows:

Urine is collected and stored either frozen or refrigerated for not more than two (2) days. Then, sodium azide is then added at a concentration
20   of 1 gram/liter and the sample is stored frozen until sufficient material is collected for the fractionation.

At this point, the urine is thawed over night, the pH adjusted to 7.2 to 7.4 with sodium hydroxide and then centrifuged to remove any precipitate (alternatively, the precipitate can be allowed to sediment,
25   e.g., for 1 hour at room temperature, approximately 75% of the supernatant is decanted, the remainder of the supernatant and the precipitate is centrifuged to pellet the precipitate, and the supernatant

80

decanted and added to the first volume of decanted supernatant). The urine is then filtered through, e.g., a 45 micron filter to remove any remaining particulate matter.

Next, the urine is concentrated using any concentration method available

5 in the art which does not remove higher molecular weight material, e.g., material larger than 3,000 daltons in molecular weight; for example, the material may be concentrated using a Pellicon (Millipore) filtration system with a membrane filter cassette having a molecular weight cut off of 3,000 daltons. Concentration with the Pellicon filtration system using

10 the 3,000 molecular weight membrane filter cut off concentrates 30 liters of urine to 500 ml (i.e., a 60-fold concentration) overnight.

To remove salts and lipids, the concentrate can then be passed over a column containing a large volume of Sephadex G25 resin in 0.05 M ammonium bicarbonate (for example, 250 ml of the concentrate can be

15 passed over a column of approximately 1.7 liters, washing the column with 25% ethanol between runs to remove adsorbed lipids and glycoprotein). The resulting desalted and delipidated urine concentrate is then lyophilized.

The lyophilized urine material or commercial hCG preparation (or any source

20 of native hCG or β-hCG) is resuspended in either phosphate buffered saline (PBS-- 30 mM sodium phosphate buffer, pH 8.3) or in 0.10 M ammonium bicarbonate at a concentration and in a volume appropriate for the column upon which the sample will be loaded, for example, but not limited to 0.5 grams of protein in 6 ml (i.e., approximately 83 mg/ml). It is within the skill of the

25 skilled artisan to determine the concentration and volume of the sample to be subjected to fractionation.

81

The sample can then be fractionated by any method known in the art for the separation of proteins. A preferred method is high resolution gel filtration on a Pharmacia pre-packed SUPERDEX™ 200 column (26/60) by HPLC using any available HPLC apparatus, e.g., with a Hewlett Packard 1050 HPLC equipped

5   with a photodiode array detector. The resuspended sample is loaded onto the column in 30 mM phosphate buffer, pH 8.3, and the material can then be eluted from the column with 30 mM sodium phosphate buffer, pH 7.0; 2M NaCl in e.g. 4 ml fractions. Fractionation can also be performed using other types of chromatography matrices e.g., heparin, DEAE-cellulose, Sephadex A50,

10 Sephadex G100, phenyl sepharose, or any sizing, ion-exchange, affinity chromatography or any other chromatography matrix available in the art. The column chromatography can also be run using any method available in the art, e.g., standard gravity flow or low pressure chromatography, FPLC, or reverse phase HPLC.

15 Many separation techniques are known in the art. Those skilled in the art would understand how to apply these known techniques to the fractionation of hCG preparations.

Once the material has been fractionated, any method known in the art, such as but not limited to, those described herein, can be used to determine which

20 fractions have anti-HIV activity and/or anti-KS activity and/or a pro-hematopoietic effect.

When fractionating by size, such as fractionation on the SUPERDEX™ 200 column, the apparent molecular weight of material in the fractions can be determined by the relative elution of those fractions compared with the elution

25 of specific hCG and β-hCG species having a known molecular weight or with the elution of known protein size markers. In general, proteins elute from a sizing column as a function of their molecular weight. The elution of, for example, hCG and the β-hCG core protein can be determined by assaying the

column chromatography fractions for the presence of hCG and the β-hCG core protein, or any hCG or β-hCG species, by any immunoassay technique available in the art, such as radioimmunoassays (either liquid or solid phase), enzyme-linked assays or ELISA assays.

5 Antibodies, either polyclonal or, preferably, monoclonal, can be generated against hCG or the β-hCG core protein by any method known in the art. Preparation of monoclonal antibodies against hCG and β-hCG species have been described in the art, see, e.g., O'Connor et al., 1994, *Endocrine Reviews* 15:650-683; Krichevsky et al, 1991, *Endocrinology* 128:1255-1264; and

10 Krichevsky et al., 1988, *Endorcrinology* 123:584-593. For the production of antibodies, various host animals can be immunized by injection with hCG, the β-hCG core protein or any other species of hCG, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not

15 limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. For preparation of monoclonal

20 antibodies, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-

25 hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Monoclonal cells lines can then be screened for binding to the particular hCG or β-hCG species using the purified species in any type of immunoassay

83

available in the art (*see, e.g.*, Erlich et al., 1985, *Am. J. Reprod Immunol. Microbiol.* 8:48).

The fractions can then be assayed for the presence of the hCG or β-hCG species using a monoclonal antibody specific for the hCG or β-hCG species. The assay can be performed by any method known in the art. For example, an immunoradiometric assay (IRMA) can be used (Krichevsky et al., 1988, *Endocrinology* 123:584-593). Briefly, the IRMA assay is performed by adsorbing an antibody against the hCG or β-hCG species onto the surface of wells of a microtiter plate by incubation in a coating buffer (0.2 M sodium bicarbonate, pH 9.5) overnight at 4°C. The residual non-specific binding sites are blocked by the addition of a 1% bovine serum albumin solution (with 0.1% sodium azide) to the wells for 3 hours at room temperature, and the wells of the microtiter plate are then washed with deionized water. An aliquot of the fraction in assay buffer (0.01 M sodium phosphate, 0.15 M NaCl, 0.01 M EDTA, 0.1% sodium azide, 0.1% bovine γ-globulin, pH 7.4) is incubated in the wells for 24 hours at room temperature. The sample is then removed and the wells washed with deionized water. A solution of a second antibody specific for the hCG or β-hCG species, which antibody has been iodinated with I^{125}, (approximately 40,000 cpm/well) is incubated in the wells for 24 hours at room temperature. The iodinated antibody solution is removed and the wells washed five times with deionized water. The level of radioactivity in each well is then determined in a scintillation counter which can measure γ-irradiation.

In a specific embodiment, a preparation comprising hCG is used that contains not only the hCG heterodimer but also peptide fragments thereof, e.g., β chain peptides.

In a preferred embodiment of the invention, proteins (e.g., peptides), the amino acid sequence of which consists of one or more portions effective to increase the production of one or more hematopoietic cell types of the β-hCG sequence

84

(β-hCG peptides) are used to treat or prevent HIV infection, cancer, wasting syndrome and/or hematopoietic deficiencies. In various specific embodiments, the portion(s) of the β-hCG sequence are at least 3, 5, 10, 20, or 30 amino acids. These proteins are preferably β-hCG peptides, or nucleic acids encoding

5   β-hCG peptides, from amino acids 41-54, 45-54, 47-53 and 45-57 (SEQ ID NOS:3-6, respectively) of Figure 8 (a portion of SEQ ID NO:2). In other embodiments, these proteins are β-hCG peptides, or nucleic acids encoding β-hCG peptides, of amino acids 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-

10   145, 7-40, 46-65, or 48-56 (SEQ ID NOS:8-25 or 33-35, respectively) of Figure 8 (a portion of SEQ ID NO:2), and preferably containing a sequence from amino acids 41-54, 45-54 or 109-119 (SEQ ID NOS:3, 4 or 7, respectively) of Figure 8 (a portion of SEQ ID NO:2), and most preferably containing a sequence from amino acids 47-53 or 45-57 of Figure 8 (a portion of SEQ ID NO:2), or circular

15   [C44V]45-57 peptide (SEQ ID NO:26), or branched 45-57 (SEQ ID NO:6) peptide, or branched circular [V44C]45-57 peptide are used to treat or prevent hematopoietic deficiencies.

In another embodiment, the invention provides proteins, the amino acid sequences of which consist of two or more at least 5, 7 or 10 amino acid, non-naturally contiguous portions of the β-hCG sequence (Figure 8 (SEQ ID NO:2))

20   linked by peptide bonds between the N-terminus of one portion and the C-terminus of another portion. Specifically, proteins, the amino acid sequences of which consist of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of a peptide of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of

25   amino acids 110-119 (SEQ ID NO:27); or an isolated protein of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 4 (portions of SEQ ID NO:2), i.e., the fused peptides denoted

30   as 45-57::109-119, 110-119::45-57, or 47-57::108-119 (SEQ ID NOS:30-32,

85

respectively). Derivatives of the foregoing fusion proteins are also provided (e.g., branched, cyclized, N- or C-terminal chemically modified, etc.). In another embodiment, fusion proteins comprising two or more such portions of the β-hCG sequence are provided; such portions may or may not be contiguous to one another (i.e., an intervening sequence may be present). Molecules comprising such portions linked by hydrocarbon linkages are also provided. In another embodiment, the peptides of the invention (i) have an amino acid sequence consisting of no more than 8 peptides of the β-hCG sequence (Figure 8 (SEQ ID NO:2)) and (ii) comprise amino acid numbers 47-53 (SEQ ID NO:5) of β-hCG (Figure 8 (SEQ ID NO:2)).

In another embodiment, a protein is used that (a) comprises one or more portions of the amino acid sequence consisting of β-hCG, a peptide having an amino acid sequence consisting of said portion(s) being effective to increase production of one or more hematopoietic cell types; and (b) lacks β-hCG amino acids contiguous to said portion(s). In another embodiment, a protein is used that (a) comprises a β-hCG amino acid sequence consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-119, 109-145, 7-40, 46-65 or 48-56 (SEQ ID NOS:3-25 or 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2) and (b) lacks β-hCG amino acids contiguous to said sequence. Peptides containing the above sequences in which only conservative substitutions have been made are also provided by the present invention, as but one example of peptide derivatives within the scope of the invention. Analogs of the above-mentioned proteins and peptides which have one or more amino acid substitutions forming a branched peptide (e.g., by substitution with an amino acid or amino acid analog having a free amino- or carboxy-side chain that forms a peptide bond with a sequence of one or more amino acids, including but not limited to prolines) or allowing circularization of the peptide (e.g., by substitution with a cysteine, or insertion of a cysteine at the amino- or carboxy-terminus or

86

internally), to provide a sulfhydryl group for disulfide bond formation, are also provided.

Other β-hCG peptides, and nucleic acids encoding these peptides, may have utility in the therapeutic methods of the invention. The utility of β-hCG
5   peptides may be determined by the *in vitro* and *in vivo* assays described in herein or by any other method known in the art.

In specific embodiments, peptides of less than 50, or less than 25, amino acids are provided.

The invention also relates to derivatives, modifications and analogs of β-hCG
10   peptides. One embodiment of the invention provides a purified derivative of a protein effective to increase the production of one or more hematopoietic cell types, which protein contains an amino acid sequence of one or more portions effective to increase the production of one or more hematopoietic cell types of β-hCG. Another embodiment of the invention provides a purified derivative of
15   a protein effective to increase the production of one or more hematopoietic cell types, the amino acid sequence of which protein is selected from the group consisting of amino acid numbers 41-54, 45-54, 47-53, 45-57, 109-119, 41-53, 42-53, 43-53, 44-53, 44-57, 45-53, 46-53, 45-54, 45-55, 45-56, 45-58, 47-54, 47-55, 47-56, 47-58, 48-145, 58-145, 109-145, 7-40, 46-65 or 48-56 (SEQ ID
20   NOS:3-25 or 33-35, respectively) as depicted in Figure 8 (a portion of SEQ ID NO:2). In another embodiment, β-hCG peptide derivatives can be made by altering the β-hCG peptide sequence by substitutions, additions or deletions that provide for therapeutically effective molecules. Thus, the β-hCG peptide derivatives include peptides containing, as a primary amino acid sequence, all
25   or part of the particular β-hCG peptide sequence including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a peptide which is functionally active. For example, one or more amino acid residues within the sequence can be

87

substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar

5   (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid

10   and glutamic acid. Such β-hCG peptide derivatives can be made either by chemical peptide synthesis or by recombinant production from a nucleic acid encoding the β-hCG peptide which nucleic acid has been mutated. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis, in $vitro$ site-directed mutagenesis

15   (Hutchinson, C., et al., 1978, $J.$ $Biol.$ $Chem$ $\underline{253}$:6551), use of TAB® linkers (Pharmacia), etc.

In addition, β-hCG peptides and analogs and derivatives of β-hCG peptides can be chemically synthesized. (See, e.g., Merrifield, 1963, $J.$ $Amer.$ $Chem.$ $Soc.$ $\underline{85}$:2149-2156.) For example, peptides can be synthesized by solid phase

20   techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 50-60). β-hCG peptides can also be synthesized by use of a peptide synthesizer. The composition of the synthetic peptides may be confirmed by amino acid analysis

25   or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 34-49). Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the β-hCG peptide. Non-classical amino acids include but are not limited to the D-isomers

30   of the common amino acids, 2,4-diaminobutyric acid, α-amino isobutyric acid,

88

4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,

5   β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

By way of example but not by way of limitation, peptides of the invention can

10   be chemically synthesized and purified as follows: Peptides can be synthesized by employing the N-α-9-fluorenylmethyloxycarbonyl or Fmoc solid phase peptide synthesis chemistry using a Rainin Symphony Multiplex Peptide Synthesizer. The standard cycle used for coupling of an amino acid to the peptide-resin growing chain generally includes: (1) washing the peptide-resin

15   three times for 30 seconds with N,N-dimethylformamide (DMF); (2) removing the Fmoc protective group on the amino terminus by deprotection with 20% piperdine in DMF by two washes for 15 minutes each, during which process mixing is effected by bubbling nitrogen through the reaction vessel for one second every 10 seconds to prevent peptide-resin settling; (3) washing the

20   peptide-resin three times for 30 seconds with DMF; (4) coupling the amino acid to the peptide resin by addition of equal volumes of a 250 mM solution of the Fmoc derivative of the appropriate amino acid and an activator mix consisting or 400 mM N-methylmorpholine and 250 mM (2-(1H-benzotriazol-1-4))-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in DMF; (5) allowing the

25   solution to mix for 45 minutes; and (6) washing the peptide-resin three times for 30 seconds of DMF. This cycle can be repeated as necessary with the appropriate amino acids in sequence to produce the desired peptide. Exceptions to this cycle program are amino acid couplings predicted to be difficult by nature of their hydrophobicity or predicted inclusion within a

30   helical formation during synthesis. For these situations, the above cycle can be

modified by repeating step 4 a second time immediately upon completion of the first 45 minute coupling step to "double couple" the amino acid of interest. Additionally, in the first coupling step in peptide synthesis, the resin can be allowed to swell for more efficient coupling by increasing the time of mixing in

5 the initial DMF washes to three 15 minute washes rather than three 30 second washes. After peptide synthesis, the peptide can be cleaved from the resin as follows: (1) washing the peptide-resin three times for 30 seconds with DMF; (2) removing the Fmoc protective group on the amino terminus by washing two times for 15 minutes in 20% piperdine in DMF; (3) washing the peptide-resin

10 three times for 30 seconds with DMF; and (4) mixing a cleavage cocktail consisting of 95% trifluoroacetic acid (TFA), 2.4% water, 2.4% phenol, and 0.2% triisopropysilane with the peptide-resin for two hours, then filtering the peptide in the cleavage cocktail away from the resin, and precipitating the peptide out of solution by addition of two volumes of ethyl ether. Specifically,

15 to isolate the peptide, the ether-peptide solution can be allowed to sit at -20°C for 20 minutes, then centrifuged at 6,000xG for 5 minutes to pellet the peptide, and the peptide can be washed three times with ethyl ether to remove residual cleavage cocktail ingredients. The final peptide product can be purified by reversed phase high pressure liquid chromatography (RP-HPLC) with the primary

20 solvent consisting of 0.1% TFA and the eluting buffer consisting of 80% acetonitrile and 0.1% TFA. The purified peptide can then be lyophilized to a powder.

In a preferred embodiment, the invention provides a peptide with branched amino acids (branched peptide), preferably a branched peptide of amino acids

25 45-57 (SEQ ID NO:6) with branches occurring at positions 47 and 51, respectively, instead of the Gly and Ala residues normally present. Most preferably, diaminobutyric acid is substituted for the gly and ala residues at positions 47 and 51, respectively, and proline bonded to both diaminobutyric acid residues (45-57 branched) as shown in Figure 9A.

90

In other specific embodiments, branched versions of the β-hCG peptides listed hereinabove are provided, e.g., by substituting one or more amino acids within the β-hCG sequence with an amino acid or amino acid analog with a free side chain capable of forming a peptide bond with one or more amino acids (and

5   thus capable of forming a "branch").

Branched peptides may be prepared by any method known in the art for covalently linking any naturally occurring or synthetic amino acid to any naturally occurring or synthetic amino acid in a peptide chain which has a side chain group able to react with the amino or carboxyl group on the amino acids

10   so as to become covalently attached to the peptide chain. In particular, amino acids with a free amino side chain group, such as, but not limited to, diaminobutyric acid, lysine, arginine, ornithine, diaminopropionic acid and citrulline, can be incorporated into a peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free amino side

15   group, from that residue. Alternatively, amino acids with a free carboxyl side chain group, such as, but not limited to, glutamic acid, aspartic acid and homocitrulline, can be incorporated into the peptide so that an amino acid can form a branch therewith, for example, by forming a peptide bond to the free carboxyl side group, from that residue. The amino acid forming the branch can

20   be linked to a side chain group of an amino acid in the peptide chain by any type of covalent bond, including, but not limited to, peptide bonds, ester bonds and disulfide bonds. In a specific embodiment, amino acids, such as those described above, that are capable of forming a branch point, are substituted for β-hCG residues within a peptide having a β-hCG sequence.

25   Branched peptides can be prepared by any method known in the art. For example, but not by way of limitation, branched peptides can be prepared as follows: (1) the amino acid to be branched from the main peptide chain can be purchased as an N-α-tert-butyloxycarbonyl (Boc) protected amino acid pentafluorophenyl (Opfp) ester and the residue within the main chain to which

91

this branched amino acid will be attached can be an N-Fmoc-α-γ-diaminobutyric acid; (2) the coupling of the Boc protected amino acid to diaminobutyric acid can be achieved by adding 5 grams of each precursor to a flask containing 150 ml DMF, along with 2.25 ml pyridine and 50 mg dimethylaminopyridine and

5 allowing the solution to mix for 24 hours; (3) the peptide can then be extracted from the 150 ml coupling reaction by mixing the reaction with 400 ml dichlormethane (DCM) and 200 ml 0.12N HCl in a 1 liter separatory funnel, and allowing the phases to separate, saving the bottom aqueous layer and re-extracting the top layer two more times with 200 ml 0.12 N HCl; (4) the

10 solution containing the peptide can be dehydrated by adding 2-5 grams magnesium sulfate, filtering out the magnesium sulfate, and evaporating the remaining solution to a volume of about 2-5 ml; (5) the dipeptide can then be precipitated by addition of ethyl acetate and then 2 volumes of hexanes and then collected by filtration and washed two times with cold hexanes; and (6) the

15 resulting filtrate can be lyophilized to achieve a light powder form of the desired dipeptide. Branched peptides prepared by this method will have a substitution of diaminobutyric acid at the amino acid position which is branched. Branched peptides containing an amino acid or amino acid analog substitution other than diaminobutyric acid can be prepared analogously to the

20 procedure described above, using the N-F-moc coupled form of the amino acid or amino acid analog.

In a preferred embodiment, the peptide is a cyclic peptide, preferably a cyclic peptide of β-hCG amino acids 44-57 (SEQ ID NO:26) with cysteine substituted for valine at position 44 and circularized via a disulfide bond between the

25 cysteine residues at positions 44 and 57 (C[V44C] 45-57) (Figure 9B) or a cyclic fused peptide of β-hCG amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus by a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and circularized via a disulfide bond between the cysteine residues at positions 110 and 57. In another preferred embodiment, the peptide is a cyclic

30 branched peptide of β-hCG amino acids 44-57 (SEQ ID NO:12) with cysteine

92

substituted for valine at position 44 and circularized via a disulfide bond between the cysteine residues at positions 44 and 57 and positions 47 and 51 substituted with a diaminobutyric acid residue on which a proline is peptide bonded to its free amino sidechain.

5   Cyclization can be, for example, but not by way of limitation, via a disulfide bond between two cysteine residues or via an amide linkage. For example, but not by way of limitation, disulfide bridge formation can be achieved by (1) dissolving the purified peptide at a concentration of between 0.1.-0.5 mg/ml in 0.01 M ammonium acetate, pH 7.5; (2) adding to the dissolved peptide 0.01 M
10   potassium ferricyanide dropwise until the solution appears pale yellow in color and allowing this solution to mix for 24 hours; (3) concentrating the cyclized peptide to 5-10 ml of solution, repurifying the peptide by reverse phase-high pressure liquid chromatography (RP-HPLC) and finally lyophilizing the peptide. In a specific embodiment, in which the peptide does not contain two
15   appropriately situated cysteine residues, cysteine residues can be introduced at the amino-terminus and/or carboxy-terminus and/or internally such that the peptide to be cyclized contains two cysteine residues spaced such that the residues can form a disulfide bridge. Alternatively, a cyclic peptide can be obtained by generating an amide linkage using, for example but not limited to,
20   the following protocol: An allyl protected amino acid, such as aspartate, glutamate, asparagine or glutamine, can be incorporated into the peptide as the first amino acid, and then the remaining amino acids are coupled on. The allyl protective group can be removed by a two hour mixing of the peptide-resin with a solution of tetrakistriphenylphophine palladium (0) in a solution of chloroform
25   containing 5% acetic acid and 2.5% N-methylmorpholine. The peptide resin can be washed three times with 0.5% N,N-diisopropylethylamine (DIEA) and 0.5% sodium diethyldithiocabamate in DMF. The amino terminal Fmoc group on the peptide chain can be removed by two incubations for 15 minutes each in 20% piperdine in DMF, and washed three times with DMF for 30 seconds
30   each. The activator mix, N-methylmorpholine and HBTU in DMF, can be

brought onto the column and allowed to couple the free amino terminal end to the carboxyl group generated by removal of the allyl group to cyclize the peptide. The peptide can cleaved from the resin as described in the general description of chemical peptide synthesis above and the peptide purified by

5   reverse phase-high pressure liquid chromatography (RP-HPLC). In a specific embodiment, in which the peptide to be cyclized does not contain an allyl protected amino acid, an allyl protected amino acid can be introduced into the sequence of the peptide, at the amino-terminus, carboxy-terminus or internally, such that the peptide can be cyclized.

10 β-hCG peptides can also be obtained by recombinant expression techniques. (See, e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d Ed., Cold Spring Harbor, New York, Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K., Vol. I, II). The nucleic acid sequence encoding hCG has been cloned and

15 the sequence determined (see Figure 8 and Xia, H., 1993, *J. Molecular Endocrinology* June 10; 1993:337-343; Sherman, G.B., 1992, *J. Molecular Endocrinology*, June 6, 1992:951-959; Gieseman, L.K. (ed.), 1991, *Basic and Chemical Endocrinology*, pp. 543-567; Ward et al., 1991, in *Reproduction in Domestic Animals*, 4th ed., P.T. Coppos, ed., pp. 25-80, Academic Press, New

20 York) and can be isolated using well-known techniques in the art, such as screening a library, chemical synthesis, or polymerase chain reaction (PCR).

To recombinantly produce a β-hCG peptide, a nucleic acid sequence encoding the β-hCG peptide is operatively linked to a promoter such that the β-hCG peptide is produced from said sequence. For example, a vector can be

25 introduced into a cell, within which cell the vector or a portion thereof is expressed, producing the β-hCG peptide. In a preferred embodiment, the nucleic acid is DNA if the source of RNA polymerase is DNA-directed RNA polymerase, but the nucleic acid may also be RNA if the source of polymerase is RNA-directed RNA polymerase or if reverse transcriptase is present in the cell

94

or provided to produce DNA from the RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or

5   others known in the art, used for replication and expression in bacterial or mammalian cells.

Expression of the sequence encoding the β-hCG peptide can be by any promoter known in the art to act in bacterial or mammalian cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the

10   SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell* 22:787-797), the HSV-1 (herpes simplex virus-1) thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1441-1445), the regulatory sequences of the metallothionein gene

15   (Brinster et al., 1982, *Nature* 296:39-42), etc., as well as the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987,

20   *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control

25   region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer et al., 1987, *Science* 235:53-58), alpha 1-antitrypsin gene

30   control region which is active in the liver (Kelsey et al., 1987, *Genes and*

Devel. 1:161-171), beta-globin gene control region which is active in erythroid cells (Mogram et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46, 89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712),

5   myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286), and gonadotropin releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378). The promoter element which is operatively linked to the nucleic acid encoding the β-hCG peptide can also be a bacteriophage promoter

10   with the source of the bacteriophage RNA polymerase expressed from a gene for the RNA polymerase on a separate plasmid, *e.g.*, under the control of an inducible promoter, for example, a nucleic acid encoding the β-hCG peptide operatively linked to the T7 RNA polymerase promoter with a separate plasmid encoding the T7 RNA polymerase.

15   In a less preferred embodiment, peptides can be obtained by proteolysis of hCG followed by purification using standard techniques such as chromatography (*e.g.*, HPLC), electrophoresis, etc.

Also included within the scope of the invention are β-hCG peptide derivatives which are differentially modified during or after synthesis, *e.g.*, by benzylation,

20   glycosylation, acetylation, phosphorylation, amidation, pegylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. In specific embodiments, the peptides are acetylated at the N-terminus and/or amidated at the C-terminus. Any of numerous chemical modifications may be carried out by

25   known techniques, including but not limited to acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In another embodiment, the β-hCG peptide derivative is a chimeric, or fusion, protein comprising a functional β-hCG peptide joined at its amino- or carboxy-

96

terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a β-hCG-coding sequence joined in-frame to a coding sequence for a different protein). Such

5   a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide

10   synthesizer.

5.4   GENE THERAPY

In a specific embodiment, nucleic acids comprising a sequence encoding β-hCG or a β-hCG peptide or fused β-hCG peptides (i.e., two or more β-hCG peptides linked at the N-termini and C-termini via peptide bond(s)), are administered for

15   treatment or prevention of HIV infection, wasting, and/or cancer, and/or promotion of hematopoiesis by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment of the invention, the nucleic acid produces its encoded protein that mediates a therapeutic effect. For example, any of the methods for gene therapy

20   available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993,

25   Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley

& Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a preferred aspect, the nucleic acid encoding β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein is part of an expression vector

5   that produces β-hCG protein or a β-hCG peptide or fused β-hCG peptides or related fusion protein in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the nucleic acid sequence coding for β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein, said promoter being inducible or constitutive, and, optionally, tissue-specific. In

10   another particular embodiment, a nucleic acid molecule is used in which the β-hCG sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of β-hCG (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra et al., 1989, *Nature* 342:435-

15   438).

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then administered to the patient. These two approaches are

20   known, respectively, as *in vivo* or ex *vivo* gene therapy.

In a specific embodiment, the nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and

25   administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or

cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the cell or nucleus, e.g., by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see e.g., Wu and Wu,

5   1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc.   In a specific embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO92/06180 dated April 16, 1992 (Wu et al.); WO92/22635 dated December 23, 1992 (Wilson et

10   al.); WO92/20316 dated November 26, 1992 (Findeis et al.); WO93/14188 dated July 22, 1993 (Clarke et al.), WO93/20221 dated October 14, 1993 (Young)). In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation.   Alternatively, the nucleic acid

15   can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

In a specific embodiment, a viral vector that contains the nucleic acid sequence encoding β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion

20   protein is used.   For example, a retroviral vector can be used (see Miller et al., 1993, Meth. Enzymol. 217:581-599).   These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome.   Retroviral vectors are maintained in infected cells by integration into genomic sites upon cell division.   The nucleic acid to be used

25   in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient.   More detail about retroviral vectors can be found in Boesen et al., 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy.   Other references illustrating the use of

30   retroviral vectors in gene therapy are: Clowes et al., 1994, J. Clin. Invest.

93:644-651; Kiem et al., 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

5 Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing 10 cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be 15 found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; and Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300.) Herpes viruses are other viruses that can also be used.

20 Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are 25 expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can

be carried out by any method known in the art, including, but not limited to, transfection, electroporation, microinjection, infection with a viral vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous

5   techniques are known in the art for the introduction of foreign genes into cells (see e.g., Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599-618; Cohen et al., 1993, *Meth. Enzymol.* 217:618-644; Cline, 1985, *Pharmac. Ther.* 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are

10   not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, recombinant blood cells

15   (e.g., hematopoietic stem or progenitor cells) are administered intravenously. Additionally, epithelial cells can be injected, e.g., subcutaneously, or recombinant skin cells (e.g., keratinocytes) may be applied as a skin graft onto the patient. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

20   In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid sequence coding for β-hCG or a β-hCG peptide or fused β-hCG peptides or related fusion protein is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or

25   progenitor cells, preferably hematopoietic stem or progenitor cells, are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention.

101

Administration of hematopoietic, preferably hematopoietic stem and progenitor, cells which have been induced to proliferate with a Therapeutic of the invention and have undergone gene therapy, *i.e.*, which have stably incorporated a heterologous gene capable of expression by their progeny cells, can be of great value in the treatment of diseases and disorders affecting cells of hematopoietic lineage. In one embodiment, hematopoietic reconstitution with such recombinant hematopoietic cells can be used in the treatment of genetic disorders of the hematopoietic system. Such genetic disorders include but are not limited to those listed in Table 1, *supra*. Genetic deficiencies or dysfunctions of hematopoietic cells can be treated by supplying, to a patient, recombinant stem and progenitor cells. In a specific embodiment, patients who have hematopoietic cells which lack a gene or have a mutant gene, can be provided stem and progenitor cells that have incorporated a functional counterpart of the deficient gene. In particular, such genes which can be subject to gene therapy include but are not limited to hemoglobin or enzymes which mediate its synthetic pathway (e.g., for treatment of anemias such as beta-thalassemia, sickle-cell disease).

In another specific embodiment, patients with infections by pathogenic microorganisms which occur in or affect a hematopoietic cell lineage can be treated with recombinant hematopoietic cells. Such recombinant hematopoietic cells can contain a heterologous gene which is expressed as a product which ameliorates disease symptoms, is toxic to the pathogen without significant detriment to the host, or interferes with the pathogen's life cycle, etc. Pathogens which cause infections which may be treated with recombinant stem cells according to this embodiment of the invention include but are not limited to lymphotropic viruses such as HIV; gram-negative bacilli such as *Brucella* or *Listeria*; the mycobacterium which cause tuberculosis, or which cause Hansen's disease (leprosy); parasites such as *Plasmodium* (the etiological agents of malaria), or *Leishmania*; and fungi (such as those that cause pneumonia and other lethal infections secondary to immunodeficiencies) (for a discussion of

102

many of these disorders, see Harrison's Principles of Internal Medicine, 1970, 6th Edition, Wintrobe, M.M., et al., eds., McGraw-Hill, New York, pp. 798-1044).

As a particular embodiment, it is possible to construct recombinant stem or
5 progenitor cells that express a sequence which is "anti-sense" to the nucleic acid of a hematopoietic cell pathogen. Such a sequence, which is complementary to the pathogen's RNA or DNA, can hybridize to and inactivate such RNA or DNA, inhibiting the function or expression of the nucleic acid and disrupting the pathogen's life cycle. As a particular example, recombinant
10 hematopoietic cells can be used in the treatment of AIDS. Recombinant stem and progenitor cells which express an anti-sense nucleic acid that is complementary to a critical region (e.g., the long-terminal repeat or polymerase sequence) of the HIV genome (Wain-Hobson et al., 1985, Cell 40:9-17) can be used for hematopoietic reconstitution for the treatment of AIDS.

15 Many methods of gene therapy are available in the art (for general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993,
20 TIBTECH 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a preferred aspect, the nucleic acid which provides a gene product desired
25 in a subject is introduced into an expression vector that produces the gene product. In particular, such a nucleic acid has a promoter operably linked to the nucleic acid sequence of interest, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment,

103

a nucleic acid molecule is used in which the sequences of interest are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the desired protein (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra

5   et al., 1989, *Nature* 342:435-438).

In an embodiment of the invention, the nucleic acid is introduced into a hematopoietic cell that is then expanded by exposure to a Therapeutic of the invention prior to administration *in vivo* of the resulting recombinant cell. Alternatively, the nucleic acid can be introduced after expansion.   Such

10   introduction can be carried out by any method known in the art, including, but not limited to, transfection, electroporation, microinjection, infection with a viral vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign

15   genes into cells (*see, e.g.*, Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599-618; Cohen et al., 1993, *Meth. Enzymol.* 217:618-644; Cline, 1985, *Pharmac. Ther.* 29:69-92) and may be used to construct the recombinant hematopoietic cells for purposes of gene therapy. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible

20   by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art.   In a preferred embodiment, recombinant hematopoietic cells are administered intravenously.   The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be

25   determined by one skilled in the art.

5.5   PREPARATION OF HEMATOPOIETIC CELLS *IN VITRO*

Sources of hematopoietic stem and progenitor cells, which cells can be induced to proliferate according to one embodiment of the present invention, include but are not limited to bone marrow, fetal and neonatal blood (preferably from

5   the umbilical cord and/or placenta), fetal liver, adult peripheral blood, neonatal thymus, and neonatal spleen. The foregoing list of sources is deemed to include cell samples (e.g., cryopreserved cells, cell lines, long-term cell cultures) derived therefrom. The source is mammalian, e.g., mouse, cow, horse, primate, monkey, and is most preferably human. Techniques for obtaining such stem and

10   progenitor cells are well known in the art. For example, in one particular embodiment, human bone marrow cells can be obtained from the posterior iliac crest by needle aspiration (see, e.g., Kodo et al., 1984, *J. Clin. Invest.* 73:1377-1384). Neonatal blood can be obtained at birth by direct drainage from the umbilical cord and/or by needle aspiration from the delivered placenta

15   at the root and at distended veins (see U.S. Patent Nos. 5,004,681 and 5,192,553). Fetal blood can be obtained, e.g., by taking it from the fetal circulation at the placental root with the use of a needle guided by ultrasound (Daffos et al., 1985, *Am. J. Obstet. Gynecol.* 153:655-660; Daffos et al., 1983, *Am. J. Obstet. Gynecol.* 146:985), by placentocentesis (Valenti, 1973, *Am. J.*

20   *Obstet. Gynecol.* 115:851; Cao et al., 1982, *J. Med. Genet.* 19:81), by fetoscopy (Rodeck, C.H., 1984, in *Prenatal Diagnosis*, Rodeck, C.H. and Nicolaides, K.H., eds., Royal College of Obstetricians and Gynaecologists, London), etc.

The method of the invention which comprises contacting hematopoietic stem and/or progenitor cells (or other hematopoietic cells) with a Therapeutic of the

25   invention, can be carried out on unseparated, partially separated, or purified cell populations, before and/or after cryopreservation (and thawing) or *in vitro* culturing of such cell populations, before and/or after introduction of a recombinant gene, and any other desired manipulations of the cells. In a preferred aspect, samples (e.g. bone marrow or adult blood or neonatal blood)

30   can be subjected to physical and/or immunological cell separation procedures

so as to enrich for hematopoietic stem and progenitor cells (e.g., prior to culturing in the presence of a Therapeutic of the invention to induce proliferation of the cells).

Various procedures are known in the art and can be used to enrich for stem and

5   progenitor cells. These include but are not limited to equilibrium density centrifugation, velocity sedimentation at unit gravity, immune rosetting and immune adherence, counterflow centrifugal elutriation, T lymphocyte depletion, and fluorescence-activated cell sorting, alone or in combination. Procedures have been reported for the isolation of very highly enriched populations of

10   stem/progenitor cells. U.S. Patent No. 5,061,620 dated October 29, 1991 discloses a method for isolation of human hematopoietic stem cells. Murine CFU-S have been purified by several groups using slightly different procedures (Visser et al., 1984, *J. Exp. Med.* 59:1576; Nijhof et al., 1984, *Exp. Cell Res.* 155:583; Bauman et al., 1986, *J. Cell. Physiol.* 128:133; Lord and Spooncer,

15   1986, *Lymphokine Res.* 5:59). Studies using human (Emerson et al., 1985, *J. Clin. Invest.* 76:1286) or murine (Nicola et al., 1981, *Blood* 58:376) fetal liver cells have yielded highly enriched progenitor cells with up to 90% of them being colony forming cells for multi-, erythroid-, and granulocyte-macrophage lineages. CFU-E have also been very highly enriched (Nijhof et al., 1983, *J.*

20   *Cell Biol.* 96:386). Purification of adult mouse marrow CFU-GM with cloning efficiencies of up to 99% in semi-solid medium has been accomplished by pretreatment of mice three days prior to sacrifice with cyclophosphamide, density separation of cells on Ficoll-Hypaque, and counterflow centrifugal elutriation (Williams et al., 1987, *Exp. Hematol.* 15:243). The resulting fraction

25   of cells contained no detectable CFU-GEMM, BFU-E or CFU-MK, but up to 10% of the cells formed CFU-S measured at day 12. These procedures, or modifications thereof, can be used.

Human stem and progenitor cells are present in the non-adherent, low density, T-lymphocyte-depleted fraction of bone marrow, spleen, and adult and cord

106

blood cells. Low density (density less than 1.077 gm/cm^3) cells can be separated by use of Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) or Percol (Broxmeyer, H.E., 1982, *J. Clin. Invest.* <u>69</u>:632-642). In this procedure, the mature cells of the granulocytic series, which are not needed for transplantation, are removed in the dense fraction which goes to the bottom of the tube. An adherence/nonadherence separation protocol can also be used for enrichment of hematopoietic stem and progenitor cells.

It is also possible to use cell separation procedures that entail immunological recognition of cells. Stem and progenitor cells can be isolated by positive or negative selection using antibodies that recognize antigenic determinants on the surface of cells. One means is to separate the cells by using monoclonal antibodies which recognize cell surface determinants on these cells, in conjunction with separation procedures such as fluorescence-activated cell sorting or panning (Broxmeyer et al., 1984, *J. Clin. Invest.* <u>73</u>:939-953). Human hematopoietic stem and progenitor cells contain antigenic determinants that are not present on all other cells, which can be used in antibody selection protocols for enrichment purposes; such antigens include but are not limited to those described *infra*.

Within the human system, several antigens have been found on stem/progenitor cells. The first antigenic system studied intensively was that of the MHC class II antigens, especially HLA-DR. This antigen has been found on CFU-GEMM, BFU-E, and CFU-GM (Lu et al., 1983, *Blood* <u>61</u>:250; Winchester et al., 1977, *Proc. Natl. Acad. Sci. U.S.A.* <u>74</u>:4012; Busch et al., 1987, *Blut* <u>54</u>:179). Several investigators have suggested that HLA-DR are not found, or are present at a low density on cells earlier than CFU-GEMM (Moore et al., 1980, *Blood* <u>55</u>:682; Keating et al., 1984, *Blood* <u>64</u>:1159).

Groups of antibodies have been used to distinguish different progenitors of the granulocyte-macrophage lineage (Ferrero et al., 1983, *Proc. Natl. Acad. Sci.*

U.S.A. <u>80</u>:4114). Type 1 CFU-GM contribute all of the peripheral blood CFU-GM, as well as a small number of bone marrow CFU-GM. They express surface antigens recognized by S3-13 and S17-25 antibodies, but not by R1B19 and WGHS-29-1 antibodies. Type 2 CFU-GM are present only in the marrow and

5 react with S3-13, R1B19, and WGHS-29-1. Culture of type 1 CFU-GM in liquid culture generates type 2 CFU-GM. These antibodies have also been used to characterize CFU-GM from patients with chronic myeloproliferative disorders (Robak et al., 1985, *Leukemia Res.* <u>9</u>:1023; Ferrero et al., 1986, *Cancer Res.* <u>46</u>:975).

10 Other antigens on human stem/progenitor cells include those reactive with the My10 (Leary et al., 1987, *Blood* <u>69</u>:953; Strauss et al., 1986, *Exp. Hematol.* <u>14</u>:879), 3C5 (Katz et al., 1985, *Leukemia Res.* <u>9</u>:191; Katz et al., 1986, *Leukemia Res.* <u>10</u>:961), RFB-1 (Bodger et al., 1983, *Blood* <u>61</u>:1006), 12-8 (Andrews et al., 1986, *Blood* <u>67</u>:842), and L4F3 (Andrews et al., 1986, *Blood*

15 <u>68</u>:1030) antibodies. The antigen recognized by L4F3 is on CFU-GM, CFU-MK, BFU-E, and CFU-GEMM, but is apparently absent from cells which generate these progenitors in suspension culture (*id.*). The antigen recognized by the My10 antibody is CD34 (Civin et al., U.S. Patent No. 4,714,680 dated December 22, 1987.) Two subsets of pluripotent hematopoietic stem cells have

20 been reported, a CD34$^+$ HLA-DR$^+$ CD38$^-$ subset and a more primitive CD34$^+$ HLA-DR$^-$ CD38$^-$ subset, with a gradual increase in CD38 expression as the hematopoietic cells proceed toward a more differentiated state (Huang and Terstappen, 1992, *Nature* <u>360</u>:745-749; Terstappen et al., 1992, *Leukemia* <u>6</u>:993-1000). The antigen recognized by another antibody, MyII, is expressed

25 on CFU-GM, but not on BFU-E or CFU-GEMM (Strauss et al., 1986, *Exp. Hematol.* <u>14</u>:935). Receptors for various lectins are also expressed on stem/progenitor cells (Nicola et al., 1980, *J. Cell Physiol.* <u>103</u>:217; Reisner et al., 1982, *Blood* <u>59</u>:360; Reisner et al., 1978, *Proc. Natl. Acad. Sci. U.S.A.* <u>75</u>:2933; Aizawa and Tavassoli, 1986, *Int. J. Cell Cloning* <u>4</u>:464).

To expand the numbers of the hematopoietic stem and/or progenitor cells, the hematopoietic stem and/or progenitor cells (or precursor cells thereof) are exposed to or contacted with a composition comprising a Therapeutic of the invention for a sufficient time period, *i.e.*, until the desired number of cells is obtained and the time period should be for as long as it is desired to keep cells self-renewing. Preferably, the cells are contacted with the Therapeutic, for example but not limited to, 200 IU/ml hCG (e.g., hCG APL) or β-hCG preparation or a fraction of a source of hCG or β-hCG or 100 μg/ml β-hCG peptide, preferably a β-hCG peptide having the amino acid sequence of amino acid numbers 45-57 or 109-119 (SEQ ID NOS:6 or 7, respectively), or circularized peptide of amino acid numbers 44-57 (SEQ ID NO:12) with cysteine substituted for valine at position 44, or a branched peptide of amino acid numbers 45-57 (SEQ ID NO:6) with diaminobutyric acid substituted for the amino acids at positions 47 and 51 with proline peptide bonded to the diaminobutyric acid residues, or a circularized branched peptide of amino acids 44-57 (SEQ ID NO:12) with cysteine substituted for valine at position 44 and with diaminobutyric acid substituted for the amino acids at positions 47 and 51 with proline peptide bonded to the diaminobutyric acid residues or a peptide having the sequence of amino acids 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an amino acid sequence of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) or the cyclic, fused peptide, having a sequence of amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and being circularized by a disulfide bond between the terminal cysteines at positions 110 and 57 , where all amino acid numbers are of the β-hCG sequence depicted in Figure 8 (a portion of SEQ ID NO:2), while under appropriate

culture conditions, for a time period in the range of 1-21 or, more preferably, 7-21 days.

The composition comprising the Therapeutic of the invention, to which the stem and progenitor cells are exposed according to the invention, optionally also
5   contains other growth factors and/or cytokines or cell culture materials, including but not limited to erythropoietin (Epo), Steel factor (SLF), IL-1, IL-3, IL-4, IL-6, IL-11, G-CSF, GM-CSF, FBS, adult PB plasma, alone or in combination. Preferably, factors are present that cause proliferation or, less preferably, differentiation of cells that are CFU-GEMM or earlier cells, e.g.,
10   IL-3, GM-CSF.

Contacting of the stem and progenitor cells with the Therapeutic preferably occurs during cell culture and thus, the Therapeutic is preferably added to the cell culture medium being used to culture the hematopoietic stem and/or progenitor cells. Such culturing can be by any method known in the art,
15   including, but not limited to, cells grown in culture dishes, test tubes, roller bottles, bioreactors (perfusion system machines wherein cells are grown on a surface with continual perfusion by medium; e.g., as sold by Aastrom Biosciences, Inc., Ann Arbor, MI), etc. Various protocols have been described for the growth in vitro of cord blood or bone marrow cells, and it is envisioned
20   that such procedures, or modifications thereof, may be employed (see, e.g. Smith, S. and Broxmeyer, H.E., 1986, Br. J. Haematol. 63:29-34; Dexter et al., 1977, J. Cell. Physiol. 91:335; Witlock and Witte, 1982, Proc. Natl. Acad. Sci. U.S.A. 79:3608-3612). The cell culture medium is supplemented to contain an effective concentration of the Therapeutic, for example but not limited to, 200
25   I.U. hCG (APL) or β-hCG preparation or 100 μg/ml of a β-hCG peptide or a fraction of a native hCG or native β-hCG preparation.

Progeny cells of hematopoietic stem and progenitor cells of can be generated in vitro; the differentiated progeny cells thus generated can be therapeutically

110

useful. For example, in one embodiment of this aspect of the invention, hematopoietic stem cells and/or CFU-GEMM progenitor cells, can be induced to differentiate into platelets. Such platelets can be used, for example, for infusion into a patient with thrombocytopenia, such as, but not limited to, the

5   ITP associated with HIV infection. In another embodiment, granulocytes can be generated *in vitro* prior to infusion into a patient. One or more of the hematopoietic progeny cells can be generated *in vitro*, allowing for the *in vitro* production of blood components. In one embodiment, the generation of differentiated blood components is accompanied by expansion of the

10 hematopoietic stem and progenitor cell pool, in order to allow for production of a greater quantity of differentiated cells. Various growth factors can be used to promote expansion and/or differentiation of hematopoietic stem and progenitor cells, such as cytokines (growth factors) including, but not limited to, G-CSF, CSF-1, IL-3, IL-5, tumor necrosis factor-β, and α-interferon. The

15 blood components which are thus produced have uses which are not limited to therapeutic uses *in vivo*. For example, such progeny cells can be used *in vitro*, e.g., for the production and isolation of hematopoietic cell products such as growth factors, antibodies, etc.

A specific embodiment of the invention relates to a method of increasing the

20 amount of hematopoietic cells, which method comprises contacting *in vitro* a non-terminally differentiated hematopoietic cell with a composition comprising an amount of a Therapeutic of the invention effective to increase proliferation of the cell, under conditions suitable and for a time period sufficient to increase the numbers of said hematopoietic cell. For example, hematopoietic cell

25 numbers can be increased by contacting a non-terminally differentiated hematopoietic cell (e.g., a cell isolated from bone marrow or blood, adult or fetal or umbilical cord blood) with a composition comprising 200 IU/ml hCG (e.g., hCG APL) or β-hCG preparation or a fraction of a source of hCG or β-hCG or 100 μg/ml β-hCG peptide, preferably a β-hCG peptide having the amino acid

30 sequence of amino acid numbers 45-57 or 109-119 (SEQ ID NOS:6 or 7,

111

respectively), or circularized peptide of amino acid numbers 44-57 (SEQ ID NO:12) with cysteine substituted for valine at position 44, or a branched peptide of amino acid numbers 45-57 (SEQ ID NO:6) with diaminobutyric acid substituted for the amino acids at positions 47 and 51 with proline peptide

5   bonded to the diaminobutyric acid residues, or a circularized branched peptide of amino acids 44-57 (SEQ ID NO:12) with cysteine substituted for valine at position 44 and with diaminobutyric acid substituted for the amino acids at positions 47 and 51 with proline peptide bonded to the diaminobutyric acid residues or a peptide having the sequence of amino acids 45-57 (SEQ ID NO:6)

10   linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or an amino acid sequence of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) or the cyclic,

15   fused peptide, having a sequence of amino acids 110-119 (SEQ ID NO:27) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 45-57 (SEQ ID NO:6) and being circularized by a disulfide bond between the terminal cysteines at positions 110 and 57, where all amino acid numbers are of the β-hCG sequence depicted in Figure 8 (a portion of SEQ ID NO:2), and

20   culturing the cell for at least ten days.

5.6   DEMONSTRATION OF THERAPEUTIC UTILITY

The Therapeutics of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. Any *in vitro* or *in vivo* assay known in the art to measure HIV infection or

25   production can be used to test the efficacy of a Therapeutic of the invention. By way of example, and not by way of limitation, one could use any of the *in vitro* or *in vivo* assays described herein.

5.6.1 HIV

In an embodiment of the invention, a method of screening a preparation comprising hCG, an hCG alpha or hCG beta chain, fragment or derivative of hCG or said alpha or beta chain, or a fraction of a source of hCG or β-hCG for
5   anti-HIV activity is provided, which assay comprises assaying said preparation or fraction for the ability to inhibit HIV replication or expression of HIV RNA or protein. In one specific embodiment, the hCG preparation or fraction is assayed by a method comprising measuring HIV-1 p24 antigen levels in cultured hematopoietic cells acutely infected with HIV-1, which cells have been
10   contacted with the preparation or fraction; and comparing the measured HIV-1 p24 antigen levels in the cells which have been contacted with the hCG preparation or fraction with said levels in cells not so contacted with the preparation or fraction, wherein a lower level in said contacted cells indicates that the preparation or fraction has anti-HIV activity. In another specific
15   embodiment, the hCG preparation or fraction is assayed by a method comprising measuring the activity of a reporter gene product expressed from a construct in which the HIV-1 LTR is operably linked to said reporter gene, wherein said construct is present in cells which have been contacted with the preparation or fraction; and comparing the measured expression of said reporter gene in the
20   cells which have been contacted with the preparation or fraction with said levels in such cells not so contacted, wherein a lower level in said contacted cells indicates that the preparation or fraction has anti-HIV activity. In another specific embodiment, the hCG preparation or fraction is assayed by a method comprising measuring HIV-1 derived RNA transcripts or HIV-1 antigen levels in
25   HIV-1 transgenic mice administered the preparation or fraction; and comparing the measured transcript or antigen levels in the mice which have been administered the preparation or fraction with said levels in mice not so administered, wherein a lower level in said administered mice indicates that the preparation or fraction has anti-HIV activity. In yet another specific
30   embodiment, the hCG preparation or fraction is assayed by a method comprising measuring SIV p27 antigen levels in the peripheral blood mononuclear cells of

113

SIV infected monkeys administered the preparation or fraction; and comparing the measured antigen levels in the monkeys which have been exposed to the preparation or fraction with said levels in monkeys not so administered, wherein a lower level in said administered monkeys indicates that the preparation or
5   fraction has anti-HIV activity.

By way of example, to assay a Therapeutic *in vitro*, one can examine the effect of the Therapeutic on HIV replication in cultured cells. Briefly, cultured hematopoietic cells (e.g., primary PBMCs, isolated macrophages, isolated CD4$^+$ T cells or cultured H9 human T cells) are acutely infected with HIV-1 using
10   titers known in the art to acutely infect cells *in vitro*, such as 10^5 TCID$_{50}$/ml. Then, appropriate amounts of the Therapeutic are added to the cell culture media. Cultures are assayed 3 and 10 days after infection for HIV-1 production by measuring levels of p24 antigen using a commercially available ELISA assay. Reduction in p24 antigen levels over levels observed in untreated controls
15   indicates the Therapeutic is effective for treatment of HIV infection.

Additionally, assays for HIV-1 LTR driven transcription are useful for testing the efficacy of Therapeutics of the invention. Specifically, a reporter gene, *i.e.*, a gene the protein or RNA product of which is readily detected, such as, but not limited to, the gene for chloramphenicol acetyltransferase (CAT), is cloned into
20   a DNA plasmid construct such that the transcription of the reporter gene is driven by the HIV-1 LTR promoter. The resulting construct is then introduced by transfection, or any other method known in the art, into a cultured cell line, such as, but not limited to, the human CD4$^+$ T cell line HUT 78. After exposure of the transformed cells to the Therapeutic, transcription from the HIV-1 LTR is
25   determined by measurement of CAT activity using techniques which are routine in the art. Reduction in HIV-1 LTR driven transcription demonstrates utility of the Therapeutic for treatment and/or prevention of HIV infection.

Exemplary tests in animal models are described briefly as follows: First, a Therapeutic of the invention is administered to mice transgenic for HIV-1, e.g., mice which have integrated molecular clone pNL4-3 containing 7.4 kb of the HIV-1 proviral genome deleted in the gag and pol genes (Dickie, P., et al.,

5   1991, *Virology* 185:109-119). Skin biopsies taken from the mice are tested for HIV-1 gene expression by RT-PCR (reverse transcription-polymerase chain reaction) or for HIV-1 antigen expression, such as expression of gp120 or NEF, by immunostaining. Additionally, the mice are examined for reduction in the cachexia and growth retardation usually observed in HIV-1 transgenic mice

10   (Franks, R.R., et al., 1995, *Pediatric Res.* 37:56-63).

The efficacy of Therapeutics of the invention can also be determined in SIV infected rhesus monkeys (see Letrin, N.L., and King, N.W., 1990, *J. AIDS* 3:1023-1040), particularly rhesus monkeys infected with SIV$_{mac251}$, which SIV strain induces a syndrome in experimentally infected monkeys which is very

15   similar to human AIDS (Kestler, H., et al., 1990, *Science* 248:1109-1112). Specifically, monkeys can be infected with cell free SIV$_{mac251}$, for example, with virus at a titer of $10^{4.5}$ TCID$_{50}$/ml. Infection is monitored by the appearance of SIV p27 antigen in PBMCs. Utility of the Therapeutic is characterized by normal weight gain, decrease in SIV titer in PBMCs and an increase in CD4^{+} T

20   cells.

Once the Therapeutic has been tested *in vitro*, and also preferably in a non-human animal model, the utility of the Therapeutic can be determined in human subjects. The efficacy of treatment with a Therapeutic can be assessed by measurement of various parameters of HIV infection and HIV associated disease.

25   Specifically, the change in viral load can be determined by quantitative assays for plasma HIV-1 RNA using quantitative RT-PCR (Van Gemen, B., et al., 1994, *J. Virol. Methods* 49:157-168; Chen, Y.H., et al., 1992, *AIDS* 6:533-539) or by assays for viral production from isolated PBMCs. Viral production from PBMCs is determined by co-culturing PBMCs from the subject with H9 cells and

115

subsequent measurement of HIV-1 titers using an ELISA assay for p24 antigen levels (Popovic, M., et al., 1984, *Science* 204:497-500). Another indicator of plasma HIV levels and AIDS progression is the production of inflammatory cytokines such as IL-6, IL-8 and TNF-α; thus, efficacy of the Therapeutic can be

5 assessed by ELISA tests for reduction of serum levels of any or all of these cytokines. Administration of the Therapeutic can also be evaluated by assessing changes in CD4$^+$ T cell levels, body weight, or any other physical condition associated with HIV infection or AIDS or AIDS Related Complex (ARC). Reduction in HIV viral load or production, increase in CD4$^+$ T cell or

10 amelioration of HIV-associated symptoms demonstrates utility of a Therapeutic for administration in treatment/prevention of HIV infection.

5.6.2 WASTING

Specific embodiments provide a method for screening a preparation comprising hCG or an hCG α chain or hCG β chain or a derivative of hCG or of said alpha

15 or beta chain or a fraction of native hCG or native β-hCG, for anti-wasting activity comprising assaying said preparation for the ability to promote weight gain in an animal model that exhibits a wasting syndrome. In one specific embodiment, the hCG preparation is screened by a method comprising measuring the weight of an offspring of an HIV-1 transgenic mouse, which

20 offspring has been exposed to the preparation; and comparing the weight of the offspring which has been exposed to the preparation with the weight of an offspring not so exposed, wherein a greater weight in said exposed offspring indicates that the preparation has anti-wasting activity. In another specific embodiment, the hCG preparation is screened by a method comprising

25 measuring the change in weight of an SIV infected monkey which has been exposed to the preparation; and comparing the change in weight of the monkey which has been exposed to the preparation to the change in weight of a monkey which has not been so exposed, wherein a greater weight increase or smaller

weight loss in said exposed monkey indicates that the preparation has anti-wasting activity.

Any animal model in which wasting occurs can be used. Exemplary tests in animal models are described briefly as follows: First, a Therapeutic of the

5   invention can be assayed in mice transgenic for HIV-1, e.g., mice which have integrated molecular clone pNL4-3 containing 7.4 kb of the HIV-1 proviral genome deleted in the gag and pol genes (Dickie, P., et al., 1991, *Virology* 185:109-119). These mice exhibit cachexia and growth retardation (Franks, R.R., et al., 1995, *Pediatric Res.* 37:56-63). A Therapeutic which reverses the

10   cachexia and growth retardation in the HIV transgenic mice is predicted to have utility for treatment or prevention of wasting syndromes.

Similarly, the efficacy of Therapeutics of the invention can also be assayed in SIV infected rhesus monkeys (see Letrin, N.L., and King, N.W., 1990, *J. AIDS* 3:1023-1040), particularly rhesus monkeys infected with SIVmac251, which SIV

15   strain induces a syndrome in experimentally infected monkeys which is very similar to human AIDS and results in weight loss in the infected monkeys (Kestler, H., et al., 1990, *Science* 248:1109-1112). Specifically, monkeys are infected with cell free SIVmac251, for example, with virus at a titer of $10^{4.5}$ TCID$_{50}$/ml and SIV infection is monitored by the appearance of SIV p27 antigen

20   in PBMCs. An increase in the weight of infected monkeys indicates that the Therapeutic has utility in the treatment of wasting syndrome.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to

25   humans, any animal model system known in the art may be used.

Once the Therapeutic has been tested in a non-human animal model, the utility of the Therapeutic can be determined in human subjects. Improvement in

wasting syndrome, *i.e.* and increase in body cell mass, can be assessed by any well known clinical techniques available in the art. For example but not limited to, measuring body weight, determination of total body potassium content, determination of intracellular water volume, bioelectrical impedance analysis,

5   anthropometrics and determination of total body nitrogen content (*see, e.g.,* Kotler, D.P. et al., 1985, *Am. J. Clin. Nutr.* 42:1255-65; Ott, M. et al., 1993, *Am. J. Clin. Nutr.* 57:15-19; Miller, T.L. et al., 1993, *Am. J. Clin. Nutr.* 57:588-592). Therapeutics, the administration of which increases body weight or cell mass, should have utility in treatment of wasting syndrome.

10   **5.6.3 CANCER**

One embodiment provides a method for screening a preparation comprising a protein, preferably a purified protein, having a sequence of one or more portions of β-hCG or a derivative, preferably in purified form, of said protein, or a fraction of a source of native hCG or native β-hCG for anti-cancer activity

15   comprising assaying said preparation for the ability to inhibit the survival or proliferation of malignant cells. In a specific embodiment, the preparation is screened by a method comprising measuring the survival or proliferation of malignant cells, which cells have been contacted with the preparation; and comparing the survival or proliferation of the cells contacted with the

20   preparation with the survival or proliferation of cells not so contacted with the preparation, wherein a lower level of survival or proliferation in said contacted cells indicates that the preparation has anti-cancer activity. In another specific embodiment, the preparation is screened by a method comprising measuring the survival or proliferation of cells from a cell line which is derived from or

25   displays characteristics associated with a malignant disorder, which cells have been contacted with the preparation; and comparing the survival or proliferation in the cells which have been contacted with the preparation with said survival

or proliferation in cells not so contacted, wherein a lower level in said contacted cells indicates that the preparation has anti-tumor activity.

Another embodiment provides a method for screening a preparation comprising a protein having a sequence of a portion of β-hCG or a derivative of said

5   protein or a fraction of a source of native hCG or native β-hCG, for anti-cancer activity comprising assaying said preparation for the ability to convert cells having an abnormal phenotype to a more normal cell phenotype. In a specific embodiment, the preparation is screened by a method comprising assessing the phenotype of cells suspected of being pre-neoplastic in culture, which cells

10   have been contacted with the preparation; and comparing the phenotype in the cells which have been contacted with the preparation with said phenotype in cells not so contacted, wherein a more normal phenotype in said contacted cells indicates that the preparation has anti-cancer activity. In another specific embodiment, the preparation is screened by a method comprising assessing the

15   phenotype of cells from a cell line which is derived from or displays characteristics associated with a pre-malignant disorder, which cells have been contacted with the preparation; and comparing the phenotype in the cells which have been contacted with the preparation with said phenotype in cells not so contacted, wherein a more normal phenotype in said contacted cells indicates

20   that the preparation has anti-cancer activity.

Yet another embodiment provides a method for screening a preparation comprising a protein having a sequence of a portion of β-hCG or a derivative of said protein or a fraction of a source of native hCG or native β-hCG, for activity in treatment or prevention of Kaposi's Sarcoma comprising assaying said

25   preparation for the ability to inhibit Kaposi's Sarcoma cell proliferation or promote Kaposi's Sarcoma cell apoptosis. In a specific embodiment, the preparation is screened by a method comprising measuring proliferation or colony formation in cultured KS Y-1 or KS-SLK cells, which cells have been contacted with the preparation; and comparing the measured proliferation or

119

colony formation in the cells which have been contacted with the preparation with said proliferation or colony formation in cells not so contacted with the preparation, wherein a lower level of proliferation or colony formation in said contacted cells indicates that the preparation has anti-Kaposi's Sarcoma activity.

In another specific embodiment, the preparation is screened by a method comprising measuring apoptosis in a Kaposi's Sarcoma tumor in an immunodeficient mouse, which Kaposi's Sarcoma tumors have been induced by injection with KS Y-1 or KS-SLK cells, and which mouse has been exposed to the preparation; and comparing the degree of apoptosis in the tumor of the mouse which has been exposed to the preparation with a tumor in a mouse not so exposed, wherein a higher in level of apoptosis in the tumor of said exposed mouse indicates that the preparation has anti-Kaposi's Sarcoma activity.

For example, *in vitro* assays which can be used to determine whether administration of a specific Therapeutic is indicated include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed to a Therapeutic. A Therapeutic which inhibits survival or growth of the malignant cells is selected for therapeutic use *in vivo*. Many assays standard in the art can be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, *fos, myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc.

In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

5   In another embodiment, cells of a patient tissue sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more normal (*i.e.*, less representative of a pre-neoplastic state, neoplastic state, malignant state, or transformed phenotype) is selected for therapeutic use. Many assays standard in the art can be used to assess whether a pre-neoplastic

10   state, neoplastic state, or a transformed or malignant phenotype, is present. For example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as

15   plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

      In other specific embodiments, the *in vitro* assays described *supra* can be

20   carried out using a cell line, rather than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic or pre-neoplastic disorder desired to be treated or prevented, or is derived from the cell type upon which an effect is desired, according to the present invention.

25   Specifically, Therapeutics can be tested for efficacy in treatment or prevention of Kaposi's sarcoma by any of the methods relating to Kaposi's sarcoma described herein or in Lunardi-Iskandar et al. (1995, Nature 375:64-68) or by any other method known in the art. Briefly, KS cell lines, KS Y-1 (*Ibid.*) or KS-

121

SLK (Siegal, B. et al., 1990, *Cancer* 65:492-498), which will produce malignant tumors in immunodeficient mice, are used to perform *in vitro* proliferation and clonogenic assays (*see, e.g.,* Lunardi-Iskandar, Y. et al., 1993,*J. Exp. Med.* 177:741-750); methods for performing such assays are well known in the art.

5   A Therapeutic which reduces proliferation or colony formation in the cultured cells can be used in the methods of the invention for treatment or prevention of KS.

Efficacy of a Therapeutic can also be determined by administration of the Therapeutic to immunodeficient mice injected with either the KS-Y-1 or KS-SLK

10   cells, which cause tumor formation in the mice, and assessment of the degree of apoptosis and angiogenesis of tumor cells after treatment with the Therapeutic. Apoptosis is detected by staining fixed tissue samples from the tumor for the presence of cells with DNA fragmentation. For example, this is accomplished by treating tissue slides from formalin-fixed tumors with terminal

15   deoxynucleotide transferase for extension of DNA ends (3' hydroxyl ends) and incorporation of digoxigenin-11-dUTP. Anti-digoxigenin antibody conjugated with the enzyme peroxidase allows detection of apoptotic cells that stain brown whereas viable cells stain blue. An increase in KS tumor cell apoptosis and a decrease in angiogenesis indicates that the Therapeutic has utility in treatment

20   of KS.

The Therapeutic can also be assessed in clinical trials in human patients suffering from KS or any other cancer. To test the efficacy of the Therapeutic in KS patients, either local, *i.e.* intralesional, or systemic administration of the Therapeutic can be used. Tumors can be examined physically for regression in

25   response to administration of the Therapeutic. Additionally, tissue biopsies can be taken from the tumors, and these tissue samples examined for apoptosis, as described above.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

5   ## 5.6.4 HEMATOPOIESIS

The Therapeutics of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. Any *in vitro* or *in vivo* assay known in the art to measure a pro-hematopoietic effect, *i.e.* the ability to induce hematopoietic cell proliferation *in vitro* or production

10   of one or more hematopoietic cell types *in vivo*, such as those described herein, can be used to test the efficacy of a Therapeutic of the invention.

A specific embodiment provides a method for screening a preparation comprising hCG or an hCG α chain or hCG β chain or a derivative of hCG or of said alpha or beta chain or a fraction of a source of native hCG or native β-

15   hCG, for pro-hematopoietic activity comprising assaying said preparation for the ability to induce an increase in hematopoietic cell numbers. In one embodiment, the preparation is screened by a method comprising measuring the number of colonies formed from hematopoietic stem or progenitor cells, which cells have been contacted with the preparation; and comparing the number of

20   colonies formed from the cells contacted with the preparation with the number of colonies formed from cells not so contacted with the preparation, wherein a higher number of colonies formed from said contacted cells indicates that the preparation has pro-hematopoietic activity. In another embodiment, the preparation is screened by a method comprising measuring the number of CD4$^+$

25   T cells in an SIV infected monkey, which monkey has been exposed to the preparation; and comparing the number of CD4$^+$ T cells in the monkey which has been exposed to the preparation with the number of CD4$^+$ T cells in a monkey not so exposed, wherein a higher number of CD4$^+$ T cells in said

123

exposed monkey indicates that the preparation has pro-hematopoietic activity.

Specifically, to assay a Therapeutic *in vitro*, one could examine the effect of the Therapeutic on proliferation of hematopoietic cells *in vitro*. For example, to

5   assay colony-forming units (a progenitor cell), briefly, the hematopoietic cells are cultured for an appropriate amount of time, such as 5 to 20 days and preferably 10 days, in the presence of (or otherwise exposed to) the Therapeutic to be tested, and then colony assays are performed to determine the number of colonies formed in comparison to the number of colonies formed by cells

10   cultured in the absence of the Therapeutic. For example, hematopoietic progenitor cells can be isolated from bone marrow or cord blood, seeded in methylcellulose in the presence of absence of the Therapeutic, and then colony numbers determined after 10 days of culture. An increase in colony numbers in cells contacted with the Therapeutic indicates that the Therapeutic has

15   activity in inducing proliferation of hematopoietic cells. Thus, for example, depending on the progenitor cell desired to be assayed, CFU-GM, CFU-GEMM, etc., assays can be done.

Therapeutics can also be tested *in vivo* for activity in increasing the numbers of hematopoietic cells. Preferably, Therapeutics are tested in animal models of

20   hematopoietic disorders before testing them in human patients. For example, but not by way of limitation, a Therapeutic can be tested in rhesus monkeys infected with SIV, particularly SIV$_{mac251}$ which induces a syndrome in monkeys similar to human AIDS (Kestler, H. et al., 1990, *Science* $\underline{248}$:1109-1112), and which are deficient in CD4$^+$ T cells. The Therapeutic to be tested can be

25   administered to the infected monkeys; then the blood or bone marrow of the infected monkeys can be examined for an increase in CD4$^+$ T cells or any other hematopoietic cell type for which the monkey is deficient. An increase in numbers of the hematopoietic cell demonstrates that the Therapeutic is useful

124

for treating diseases and disorders associated with hematopoietic deficiencies. Any animal model of an anemia can be similarly used for testing.

Therapeutics can be tested in human patients, preferably after tests *in vitro* and/or *in vivo* in an animal model, with hematopoietic deficiencies, for example but not limited to, deficiencies associated with HIV infection such as anemia, neutropenia, thrombocytopenia, or $CD4^+$ T cell lymphocyte deficiency, for activity in increasing numbers of hematopoietic cells for which the patient is deficient. Briefly, the Therapeutic is administered, for example by intramuscular injection two to three times per week, to the patient suffering from the hematopoietic deficiency. The subject's blood or bone marrow is assayed before and after treatment with the Therapeutic for an increase in the hematopoietic cell numbers. Therapeutics which cause an increase in hematopoietic cell numbers are useful for treatment of diseases and disorders associated with hematopoietic deficiencies.

Assays for hematopoietic cell proliferation in the blood or bone marrow can be accomplished by any method well known in the art. For example, blood can be drawn and blood cell numbers can be determined by routine clinical laboratory tests for red blood cells, platelets, neutrophils, lymphocytes, etc. Additionally, colony assays on isolated bone marrow can be performed to assess increases in stem or progenitor cells. For example, bone marrow can be sampled and bone marrow cells evaluated for stem and progenitor cell colony formation. Briefly, cells are seeded in methylcellulose, cultured for 12 to 14 days, and then scored for colony formation where aggregates containing more than 50 cells are counted as a colony (see, e.g., Lunardi-Iskandar, Y. et al., 1995, *Nature* 375:64-68; Louache, R. et al., 1992, *Blood* 180:2991-2999; Lunardi-Iskandar, Y. et al., 1989, *J. Clin. Invest.* 83:610-615). Bone marrow progenitors which can be evaluated by this colony assay include, but are not limited to, CFU-Mix, BFU-e and CFU-GM. As an alternative to colony assays for detection and quantitation of stem and/or progenitor cells, immunological detection methods can be

employed, based on the antigens expressed by the particular cell type (*see, e.g.*, the relevant discussion hereinabove).

5.7 THERAPEUTIC COMPOSITIONS AND METHODS OF ADMINISTRATION

5 The invention provides methods of treatment and prevention by administration to a subject in need of such treatment of a therapeutically or prophylactically effective amount of a Therapeutic of the invention. The subject is preferably an animal, including, but not limited to, animals such as monkeys, cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most

10 preferably human. In a specific embodiment, the subject is a human not afflicted with a cancer which secretes hCG or hCG fragments and, more particularly, not afflicted with KS.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the Therapeutic,

15 microcapsules, recombinant cells capable of expressing the Therapeutic, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous,

20 subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

25 In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed,

126

e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may
5   be achieved, for example and not by way of limitation, by topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

In another embodiment, the Therapeutic can be delivered in a vesicle, in
10   particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid*., pp. 317-327; *see generally ibid*.)

In yet another embodiment, the Therapeutic can be delivered in a controlled
15   release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida
20   (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); *see also* Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled
25   release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release, supra*, vol. 2, pp. 115-138 (1984)). Other

127

controlled release systems are discussed in the review by Langer (*Science* $\underline{249}$:1527-1533 (1990)).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered by gene therapy

5   methods as described herein.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the

10   Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the Therapeutic is administered. Such pharmaceutical carriers can be sterile

15   liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable

20   solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like.

The composition, if desired, can also contain minor amounts of wetting or

25   emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

128

The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of

5 suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

10 In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local

15 anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be

20 administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms.

25 Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived

from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vivo* and/or *in vitro* assays may optionally be employed to help predict optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Doses of, for example but not limited to, at least 15,000 I.U. and up to 45,000 I.U. hCG weekly was effective and well tolerated in humans. Weekly doses of 6,000 I.U. in monkeys and 300-500 I.U. in mice were also effective. Predicted suitable doses of a β-hCG peptide for treatment or prevention of HIV infection include, but are not limited to, 1 to 1000 micrograms per week. Routes of administration of a Therapeutic include, but are not limited to, intramuscularly, subcutaneously or intravenously. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

6 EXAMPLES

6.1 EFFECTS OF hCG, β-hCG AND β-hCG PEPTIDE PREPARATIONS ON HIV-1 INFECTION AND DISEASE

5   As described herein, we have observed beneficial effects of some preparations of human Chorionic Gonadotropin (hCG) and β-hCG against HIV disease including anti-tumor (Kaposi sarcoma, KS), anti-viral, increase in weight and pro-hematopoiesis effects. Our studies document that the same preparations inhibit KS cell growth *in vitro* and induce apoptosis in a mouse model, inhibit

10   HIV acute infection *in vitro*, down regulate HIV gene expression in 30 of 30 HIV-1 transgenic mice, inhibit SIV replication in 3 of 3 SIV acutely infected macaque monkeys with no evidence of viral resistance, promote normal hematopoiesis (including $CD4^+$ T cell rise), and reverse the wasting seen in these animals. Examples of these effects were also noted in some HIV-positive

15   patients treated with some hCG preparations. The strength of these effects varied among crude hCG preparations, and highly purified hCG did not retain these activities. However, anti-KS, anti-viral, and pro-hematopoietic effects were mimicked by native β-hCG and synthetic peptides of the beta subunit of hCG having amino acid sequences of amino acid numbers 45-57 (SEQ ID NO:6),

20   109-119 (SEQ ID NO:7), circularized 44-57, where cysteine is substituted for the amino acid at position 44 (SEQ ID NO:26), and peptides of amino acid numbers 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID

25   NO:27); or a peptide of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2). The peptides having an amino acid sequence of amino acid numbers 7-45, 47-

131

55, 46-65, and 48-56 (SEQ ID NOS:21 and 33-35, respectively) of β-hCG (Figure 8 (SEQ ID NO:2) also exhibit activity in *in vitro* assays.

6.2 EFFECTS OF hCG PREPARATIONS ON HIV-1 TRANSGENIC MICE

The HIV-1 transgenic mice used for this study contain 7.4 kb of foreign DNA,

5 including 5.1 kb of the HIV-1 proviral genome deleted in the gag and pol genes and 2.3 kb of vector (Dickie et al., 1991, *Virology* 185:109-119). The birth weights of mice homozygous for the HIV-1 transgene are normal, but soon the mice uniformly display severe growth retardation (Figure 1A), cachexia, and early mortality from expression of HIV-1 genes (env and regulatory genes) which

10 are highly expressed shortly after birth in homozygotes (Franks et al., 1995, *Pediatric Res.* 37:56-63) (Figure 1B). In addition, these mice develop severe hyperkeratotic skin lesions with marked expression of gp120 and nef genes (Kopp et al., 1993, *AIDS Res. Hum. Retroviruses* 9:267-275; Vasli et al., 1994, *AIDS Res. Hum. Retroviruses* 10:1241-1250).

15 To examine the effects of hCG preparations on HIV transgenic Tg26 mice, the mothers of 30 neonatal mice were administered a commercial preparation of native hCG (APL™, Wyeth Ayerst) (300-500 I.U.), and the mothers of other HIV-1 transgenic mice received other commercial native hCG preparations (PREGNYL™ and Sigma (G10), α-chain preparations and partially purified native

20 β-hCG and recombinant β-hCG preparations (all Sigma) (50-100 µg). For studies involving synthetic peptides, heterozygous transgenic mothers of 6 homozygous transgenic mice were given 10 µg of β-hCG peptide 45-57 (SEQ ID NO:6) where the amino acid residues at positions 47 and 51 are substituted by a branch, where the branches are made up of diaminobutyric acid and proline (branched

25 β-hCG 45-57) (prepared by Dr. N. Ambulos, UMAB) subcutaneously, daily for 10 days. Other studies were carried out with other β-hCG peptides (see Table 3).

132

Heterozygous transgenic mothers were treated with the hCG preparation subcutaneously twice weekly. Pups received hCG via the mother's milk. Blood levels of hCG in the mother and pups were monitored and ranged from 5 IU/ml to over 150 IU/ml over a 96 hour time period (data not shown).

5   Gene expression was assayed in total RNA extracted from the skin of Tg26 mice with RNAzol. One microgram of RNA was reverse transcribed into cDNA using random hexamer primers and MMTV reverse transcriptase (Life Technologies, MD) in a final volume of 30 µl. One tenth of the cDNA reactions were used for PCR amplification of various HIV gene sequences (env, tat, rev, nef and vif).

10   In addition, glyceraldehyde 3-phosphate dehydrogenase (GAPHD) mRNA was amplified for each sample for normalization. Following 25 cycles of amplification, 10% of the PCR product was resolved by electrophoresis through 2% agarose gels and processed for Southern hybridization using FITC-labeled oligonucleotide probes complementary to internal sequences of the amplicons.

15   Detection was performed by chemiluminescence (Amersham) and relative mRNA levels determined by densitometry after normalization with GAPDH mRNA levels.

The hCG (APL™) treatments resulted in marked down regulation of HIV-1 gene expression in skin biopsies as determined by the RT-PCR technique (Figure 1D).

20   The 25 cycles of amplification employed in these experiments readily detected abundantly expressed genes (e.g., env and rev) while the tat gene in treated animals was at low levels, and more readily detected with more cycles of amplification (not shown). Other skin biopsies were examined for HIV viral proteins using mouse monoclonal antibodies against gp120 and Nef by an

25   immunostaining technique. A marked decrease in viral proteins occurred after 2 weeks treatment and no detectable HIV proteins were found after 30 days of hCG treatment (not shown) and the hyperkeratosis of the skin regressed. When

the treatment was halted, reappearance of viral protein expression occurred after 2 weeks (not shown).

Associated with the decrease in HIV-1 gene expression (Figure 1D) was a reversal of the growth retardation and cachexia (Figure 1C). As described
5 immediately below, native β-hCG and some synthetic peptides of the β-subunit also reversed the adverse effects of the viral genes in these transgenic animals (Table 3). In contrast, native α-hCG had no effect on HIV gene expression or the retarded postnatal growth and cachexia (Table 3).

Table 3 and Figures 1A and B also provide results from the administration of
10 various β-hCG peptides and β-hCG peptide derivatives, *i.e.* the peptides of amino acids 45-57 (SEQ ID NO:6) ("Satellin A1"), circularized 44-57 with cysteine substituted at position 44 (SEQ ID NO:26) ("Satellin A2"), 47-57 linked at the C-terminus via a peptide bond to the N-terminus of 108-119 (SEQ ID NO:32) ("Satellin A1/B^G"), 45-57 linked at the C-terminus via a peptide bond to
15 the N-terminus of 109-119 (SEQ ID NO:30)("Satellin A1/B"), 41-54, 6-16, 47-55 (SEQ ID NO:20), and 48-56 (SEQ ID NO:35). All animals born to HIV-1 transgenic mothers which did not receive preparations containing β-hCG peptide or derivative thereof died within 10 days, showed high level of gp120 and nef protein as measured by antibody staining, and exhibited characteristic
20 hyperkeratosis. The pups receiving the β-hCG peptides 45-57, circularized 44-57 with cysteine substituted at position 44, 47-57 linked by peptide bond to 108-119, 45-57 linked by peptide bond to 109-119 (SEQ ID NOS:6, 26, 32 and 30, respectively) all exhibited considerable inhibition of HIV-1 transcription and protein expression and higher weight gain than untreated mice. The peptides
25 47-55 and 48-56 (SEQ ID NOS:20 and 32, respectively) also elicited inhibition. The scrambled peptide 45-57::109-119 and the scrambled circularized 44-57, administered as controls, showed no inhibitory effect while, surprisingly, the scrambled 45-57 peptide did exhibit some inhibition (Table 3).

Table 3

ACTIVITIES OF hCG AND hCG SUBUNIT PREPARATIONS AND hCG PEPTIDES.

Sources	Inhibition				Enhancement
	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
hCG preparations					
APL™	+++	+++	+++	+++	+++
PREGNYL™	++	++	++	++	++
ORGANON	-	ND	-	-	ND
PROFASI™	+	ND	+	+	+
GOLDLINE	+	ND	-	-	-
STERIS™	++	ND	++	ND	++
SHEIN	+	ND	-	-	-
SIGMA	+++	+++	+++	+++	+++

135

Sources	Inhibition				Enhancement
	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
SIGMA[2]	-	ND	-	-	-
CR127	-	ND	-	ND	-
CR1XY17V	-	ND	-	ND	-
CR1XY17B	-	ND	-	-	-
rhCG	-	-	-	-	-
hCG subunits					
α Chain	-	-	-	-	-
αhCG	-	-	-	-	-
rαhCG	-	-	-	-	-
αfp1769A	-	ND	-	ND	-
β Chain					

136

Sources	Inhibition				Enhancement
	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
rβhCG	-	-	-	-	-
βhCG	++	++	++	++	++
Synthetic peptides β-chain hCG					
1. 109-119	+	ND	+	+	+
2. 109-145	+	ND	+	+	+
3. 45-57	++	++	++	++	++
4. Circ 44-57	+++	+++	+++	+++	+++
5. 47-57::108-119	++	++	++	+++	ND
6. 45-57::109-119	++	++	++	+	++
7. 45-57+109-119	++	ND	++	ND	++
8. 41-54	-	-	-	-	-

137

Sources	Inhibition		Enhancement		
	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
9. 38-57	-	ND	-	-	-
10. Scrambled 45-57::109-119	-	-	-	-	-
11. Scrambled 45-57	++	ND	++	ND	ND
12. Scrambled circ. 44-57	-	ND	·	ND	-
13. 6-16	-	·	·	·	·
14. 1-20	-	ND	-	ND	ND
15. 20-47	—	ND	—	ND	—
16. 31-50	-	ND	·	ND	·
17. 46-65	+	ND	+	ND	ND

138

| Sources | Inhibition | | | | Enhancement |
	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
18. 91-112	ND	ND	-	ND	-
19. 93-100	-	ND	-	ND	ND
20. 110-145	ND	ND	-	ND	-
21. 74-95	-	ND	-	ND	-
22. 7-40	+	ND	+	ND	-
23. 57-93	-	ND	-	ND	-
24. 34-39	-	ND	-	ND	ND
25. 123-145	-	ND	-	ND	ND
26. 134-144	-	ND	-	ND	-
27. 100-110	-	ND	-	ND	ND
28. 113-132	ND	ND	-	ND	-

139

| Sources | Inhibition | | | | Enhancement |
	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
29. 128-145	-	ND	-	ND	-V
30. 37-55	+	+	+	+	+
31. 51-59	-	ND	-	ND	-
32. 48-56	+	+	+	+	+
33. Trimers	-	ND	-	ND	-
Synthetic peptides α-chain hCG					
34. 88-92	-	ND	-	ND	ND
35. 1-15	—	ND	-	-	-
36. 16-30	-	ND	-	ND	-
37. 26-45	-	ND	-	ND	-
38. 41-61	-	ND	-	ND	ND

140

Sources	HIV in vitro	HIV transgenic mice	KS in vitro	KS in vivo	Pro-hematopoiesis in vitro
	Inhibition				Enhancement
39. 57-76	-	ND	-	ND	ND
40. 72-92	-	ND	-	ND	-
41. 1-95	-	-	-	-	-

141

In Table 3, "-" represents less than 10% effect; "+" represents greater than 15% effect; "++" represents greater than 40% effect; "+++" represents greater than 70% effect; and "ND" represents no data available. The "HIV: in vitro" column reports results from assays of the inhibition of HIV-1 replication in vitro (HIV-1 strains and HIV-1 primary isolates) as described herein. The "HIV transgenic mice" column reports data from the inhibition of HIV RNA and protein expression in HIV-1 transgenic mice as described herein. Columns labeled "KS:in vitro" and "KS:in vivo" report on the inhibition of Kaposi's Sarcoma cell growth in vitro in cultured cells and of Kaposi's Sarcoma induced in mice, respectively, as described herein. Column 5 provides data on the relative increase of hematopoietic colony cell number in vitro clonogenic assays as described herein. The commercial hCG preparations tested were APL™ (Wyeth Ayerst), PREGNYL™ (Organon), ORGANON (a highly purified preparation obtained from Organon) PROFASI™ (Serono), Goldline, STERIS™, and Shein, and two preparations from Sigma, Sigma¹ (GHO) and Sigma² (C1063). The hCG preparations CR127 and CR1XY17V are highly purified hCG preparations and CR1XY17B is a mixture of highly purified α-hCG and β-hCG, all three preparations were obtained from the National Institute of Child Health and Human Development (NICHD) at the National Institute of Health (NIH) and the rhCG is recombinant hCG expressed in a mouse cell line (Sigma). For the hCG subunits "αhCG" and "βhCG" are purified native subunits (Sigma); "rαhCG" and "rβhCG" all the recombinant subunits expressed in mouse cells (Sigma); and αfp1769A is purified, native α subunit (NICHD, NIH). The peptide "scrambled A1" has the sequence Cys-Val-Ala-Gln-Pro-Gly-Pro-Gln-Val-Leu-Leu-Val-Leu-Cys (SEQ ID NO:36) and "Scrambled A2" has the sequence Cys-Val-Ala-Gln-Gly-Val-Leu-Pro-Ala-Leu-Pro-Gln-Val-Val-Cys (SEQ ID NO:37). "Scrambled A1/B" has the sequence of β-hCG amino acids 45-57 (SEQ ID NO:6) and 109-119 (SEQ ID NO:7) which has been scrambled. "Trimers" is a mixture of tripeptides from the β-hCG sequence of amino acids 45-57: Leu-Gln-Gly, Leu-Gln-Pro, Gln-Gly-Val, Gln-Pro-Val, Gln-Val-Leu, Val-Leu-Pro, Leu-Pro-Ala, Leu-Pro-Pro, Pro-Ala-Leu, Pro-Pro-Leu, Ala-Leu-Pro, Pro-Leu-Pro, Leu-Pro-Gln, Pro-Gln-Val, Gln-Val-Val,

143

and Val-Val-Cys (SEQ ID NOS: 38-53, respectively). Peptides were synthesized by Dr. N. Ambulos (University of Maryland Biomedicine Center), Becham (CA) or Peptide Technologies Corp. (Gaithersburg, MD).

5 Considerable pro-hematopoietic activity was found with the native partially purified hCG heterodimer and whole β-chain, however, variability in the pro-hematopoietic effect was observed for different hCG preparations and no pro-hematopoietic activity was observed with highly purified (to homogeneity) hCG heterodimer *in vitro*. The lower molecular weight species may retain the pro-hematopoietic effect and that some purification procedure may not eliminate

10 those species.

The available clinically used native hCG and native β-chain preparations are not homogenous and may be contaminated with one or more other active molecules. In this respect, it is noteworthy that though the effects of some preparations of hCG described here were obtained with two different commercial sources of hCG (APL and Pregnyl), one was usually more active (APL) at lower

15 concentrations than any other preparation. The differences in activities of commercial preparations might be explained by variation in the amount of β-hCG fragments. This could be the consequence of different methods of preparation or different sources of human urine. For example, free β is more abundant in the earliest weeks of pregnancy. Consequently, we initiated studies

20 with a variety of synthetic peptides, and our results show that all the *in vitro* activities of the preparations of native hCG are mimicked by the β-hCG peptides 45-57 and 109-119 but not other β- or α-peptides or scrambled 45-57 peptide. Thus, β-hCG may contain structural motifs that produce effects which probably

25 work by mechanisms which differ from those currently known for hCG, and β-hCG peptides may have biological functions quite distinct from the conventional effects of the heterodimer.

6.3 EFFECTS OF hCG PREPARATIONS IN SIV INFECTED RHESUS MONKEYS

Events early in HIV infection are thought to be critical to subsequent AIDS pathogenesis. Although the early events in HIV infection are difficult to study

5 in humans, they can be readily investigated in the SIV infected rhesus monkey animal model (Letvin et al., 1990, *J. AIDS* $\underline{3}$:1023-1040). SIV and HIV-1 are similar in many of their biological and physical properties including their genomic structure. In addition, SIV_{mac251}, unlike several other SIV isolates, induces a syndrome in experimentally infected rhesus macaques that is similar

10 to human AIDS (Kestler et al., 1990, *Science* $\underline{248}$:1109-1112).

The effect of the same commercially available hCG preparation (APL™, Wyeth Ayerst), which had been prescreened for anti-viral and anti-KS activity, was studied in five adult male rhesus monkeys who were intravenously inoculated with cell free SIV_{mac251} ($10^{4.5}$ $TCID_{50}$/ml). In all animals, SIV p27 was apparent

15 in plasma 14 days after infection, reaching a maximum by about day 20 (not shown). Treatment with systemic injections (3,000 IU, 2 times weekly) of the active commercial preparation of hCG (APL™), was initiated 3 weeks after SIV inoculation. Two months post-inoculation, the characteristic increase of SIV p27 antigen (Figure 2A), reduction of $CD4^+$ T cells (Figure 2B), and weight loss

20 (Figure 2C) occurred in 2 of 2 untreated infected monkeys. In contrast, the 3 infected monkeys treated with this hCG preparation showed weight gain characteristic of uninfected animals of this age (Figure 2C), a marked decrease in SIV p27 (Figure 2A) and an increase in $CD4^+$ T cells to normal levels (Figure 2B). These effects were maintained over the 7 months the animals were

25 followed. These results show that this commercially available hCG preparation can control SIV_{mac251} acute infection, increase $CD4^+$ T cells, and promote weight gain in SIV infected rhesus monkeys and that these effects can be maintained. The animals were followed for 7 months, and no evidence of disease or SIV resistance to the hCG preparation developed.

In Figure 2D, results are shown from 4 uninfected controls: 2 received the hCG preparation and 2 received the diluent without the hCG. There is a slight increase in the CD4$^+$ T cells in the treated animals (increasing from 460 mm^3 to 520 mm^3 and from 470 mm^3 to 650 mm^3) (Figure 2D). The 2 treated animals

5   also showed a 1 to 2 kg weight gain (not shown).

6.4   EARLY STUDIES OF SOME hCG PREPARATIONSIN PATIENTS WITH HIV-1 DISEASE

The incidence of KS is greatly increased in HIV-infected persons (Friedman-Kien et al., 1981, *J. Am. Acad. Dermatol.* 5:468-473). Based on experimental studies

10   of the killing effect of some hCG preparations on KS Y-1 cells, clinical trials with some commercially available preparations of hCG given either intralesionally (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269, Harris, P.J., 1995, *The Lancet* 346:118-119) or systemically to KS patients have shown that

15   cutaneous KS lesions were reduced via cell killing by apoptosis following intralesional inoculation (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med* 335: 1261-1269) and induced regression of advanced KS disease treated by systemic delivery.

20   Clinical trials reported herein were undertaken in Belgium and California to evaluate the anti-KS properties of systemic hCG therapy with or with out concomitant intralesional therapy. Use of anti-viral protease and non-protease inhibitors was not restricted. A total of 47 patients were enrolled under protocols of compassionate use sanctioned by the Institutional Review Boards

25   of the respective centers. 29 patients were treated in Belgium, either on a protocol to investigate intralesional and systemic treatment of cutaneous KS (n = 15), or in the pre-clinical phase of that protocol (n = 4), or on compassionate use for systemic KS or HIV infection (n = 10). The protocol involved intralesional administration of 500 IU hCG (PREGNYL^{TM}) to 4 lesions for 2

146

weeks, followed by subcutaneous administration of 2,500 IU hCG (PREGNYL™) 5 days per week for 4 to 6 weeks. Additional systemic intramuscular or subcutaneous hCG treatment with either PREGNYL™, APL™, or STERIS™ (one patient) was provided as ongoing therapy in some patients or as part of

5   compassionate use protocols.

A total of 18 patients were treated in California with at least 1 month of follow-up as part of an ongoing protocol to evaluate systemic hCG therapy for cutaneous KS. These patients received either 5000 IU of APL™ subcutaneously 7 days per week, 10,000 IU subcutaneously 3 times per week, or 10,000 IU

10   subcutaneously 7 days per week.

Overall 30 patients were on pre-existing, anti-viral therapy (19 on RT inhibitors and 11 on protease inhibitors), 11 were on no anti-virals and 8 were missing information. One patient, PH-RF, was on 3TC therapy before hCG therapy, and despite poor compliance, had an hCG response for visceral KS and viral load,

15   which declined to undetectable on hCG alone.

Thirty-six patients survived the study, 7 (PH-LFA, PH-DD, PH-PJ, PO-BO, PO-RB, PH-JJ, PH-MH) died either from opportunistic infections or multiple organ failure. The vital status of 1 patient is unknown. Two patients, PH-DD and PH-OJ, discontinued hCG treatment because of cholestasis. PH-DD was on

20   concomitant anti-mycobacterial therapy which was felt to be a contributing factor. PH-OJ had preexisting cholestasis, which was exacerbated by the hCG treatment with a marked increase in alkaline phosphatase and rise in bilirubin which required hospitalization (PH). These values declined by 2-fold following discontinuation of hCG therapy. These cases raise the possibility that liver

25   toxicity may be a rare complication of hCG therapy.

Early clinical experience with relatively low dose intralesional hCG administration for KS indicated partial or complete regression of treated lesions,

147

including 3 of the first 4 patients in the initial pilot study in Belgium (Hermans et al.,1995, *Cellular and Molecular Biology* 3:357-364) as well as a dose dependent effect between 16% (250 IU) and 83% (2,000 IU) in patients reported from California (Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269), and other

5 cases showing striking clearance of visceral (lung and gastrointestinal) KS in very advanced disease following systemic therapy with hCG APL™ or PREGNYL™ within 1 to 3 months of initiating therapy.

Among the 30 cases with cutaneous Kaposi's Sarcoma, 12 were treated with intralesional followed by systemic therapy in Belgium and 18 with systemic

10 therapy only in California. Complete (2/12, Belgium; 2/18, California) and partial (5/12, Belgium; 4/18, California) responses were observed while progressive disease was noted among 2/12 from Belgium and 10/18 in California. The overall response rate for the study (CR + PR) was 43% (13/30). The response rate in the group administered hCG both intralesionally plus

15 systemicly group was 58%, while the response rate was 33% in the group receiving only the systemic treatment. Among 8 patients with both visceral and cutaneous KS treated in Belgium with very advanced pulmonary or gastric lesions, 3 patients experienced complete remissions, 2 patients exhibited tumor stabilization and 3 progressed, in each case after failure of conventional

20 cytotoxic therapy.

AIDS patients treated with hCG therapy were tested for increases in CD4$^+$ T cell levels (in numbers of cells per mm^3) and decrease in viral load by one of the following assays for determining viral load: NASBA (Louache, et al., 1992, *Blood* 180:2991-2999; Geller, et al., 1985, *Archs. Path. Lab. Met.* 109:138-145),

25 which has a lower detection limit of 4,000 copies; Roche Amplicor, with a lower detection limit of 200 copies; RT-PCR, with a lower detection limit of 100 copies; or TCID assay in which the infection of PBMCs in co-culture is determined (Popovic et al., 1984, *Science* 204:497-500). As viral load was assayed retrospectively, the viral load results were not a factor in guiding

148

choice of therapy or changes in therapy. Each patient served as their own control and change in viral load (0.7 log change between baseline and subsequent post hCG viral load, scored as significant) was the endpoint measurement for this analysis. For analysis of the anti-viral effect, in addition

5 to the 10 patients undergoing with synchronous hCG and other anti-viral therapy, 6 patients were excluded because of a lack of base line viral load or insufficient follow up before hCG therapy was stopped or additional anti-viral therapy was started.

Among the 16 cases, 1 (PH-OJ) experienced a fall in viral load of 0.7 log on 2

10 successive tests at least 1 month apart while on stable anti-viral therapy (see Figures 3A and B), 11 were non responding and 2 (PH-VE and PHGRX) manifested an increase in viral load of at least 0.7 log after hCG therapy on 2 successive tests at least 1 month apart. As illustrated in Figures 3C and D, another patient (PG-1), initially on hCG alone and classified as non responsive

15 by study criteria (2 consecutive values of 0.7 log decrease in viral load over 1 month) on hCG alone, experienced a steady decline in viral load but the second qualifying >0.7 log viral load drop was measured 2 weeks after non protease inhibitor therapy was begun. Because of this short window, it is likely that this second stable viral load point is accounted for by hCG rather than the

20 newly introduced anti-virals. It is noteworthy that $CD4^+$ T cell levels were not significantly altered in this case but, the patient's KS progressed, documenting a dissociation of various hCG effects.

Among the 6 cases being treated with hCG alone (i.e. without other anti-viral therapies) with analyzable data, all were scored as non responsive to the hCG

25 therapy by the scoring criteria although one case (PG-1) noted above (and illustrated in Figures 3C and D) is a probable responder. An additional patient on hCG alone (PG-8; Figures 3E and F) experienced a sustained fall in viral load of 0.5 log over a 2.8 month period of treatment on hCG alone until KS lesions progressed, at which time hCG therapy was discontinued. Thus of the 7

149

analyzable patients on hCG alone, 4 exhibited a downward trend in viral load, 2 patients showed an increase in viral load, and 1 patient was stable.

To more fully evaluate all data from patients on hCG alone or with stable antiviral therapy, all eligible data points were plotted, as shown in Figure 15A,
5   indicating the coordinates for each data point pre and post therapy, with values on the line representing no change in viral load. Values are distributed more or less equally above and below the line with no obvious trend to suggest a strong anti viral effect. To evaluate a dose response relationship between hCG and viral load, regression analysis for patients on hCG, alone or with stable
10   antiviral therapy is shown in Figure 15C. There was no detectable effect of higher hCG dose on viral load level ($r = -.089$, $p = 0.285$, $N = 147$ measurements). An analysis by different CD4 strata did not show any significant trends to suggest that level of immunity impacted the hCG effect.

Among the 22 patients with analyzable CD4$^+$ T cell data, 5 demonstrated a pro-
15   CD4$^+$ T cell effect (PH-VE, PH-RF, PG-9, PG-17, and PG-19) characterized by a 50% rise in CD4$^+$ T cell count sustained over at least a one month period, as demonstrated by plotting the data from at least two patients (PH-VE--Figures 3G and H and PG-17--Figures 3I and J). Of these 5 patients, concomitant stable non protease anti-virals were administered to 2 patients, stable protease inhibitors
20   in 2 cases and hCG preparation alone in 1 case. Thus of the 6 cases with valid CD4$^+$ T cell data on hCG preparation alone, 1 manifested a significant response. No patient experienced an adverse fall in CD4$^+$ T cell on hCG preparation therapy, although patient PH-VE experienced an 0.7 log rise in viral load with a sustained 50% fall in CD4$^+$ T cell numbers and a partial anti KS response
25   (Figures 3G and H). Similarly, paient PG-17 experienced a significant rise in CD4$^+$ T cells and no change in viral load on hCG therapy alone, yet experienced progression of KS after 2.5 months (Figures 3I and J). All CD4$^+$ T cell values (except for 2 patients on hCG alone) were at or above baseline, with the most significant rises in those on concomitant stable protease inhibitor or

non protease drugs (Figure 15B). There is no correlation between a change in the CD4$^+$ T cells count and the dosage of hCG (r = .101, p = .339, N = 92) (data not shown).

Among the 26 patients analyzable for weight gain (patients who started hCG
5   preparation therapy coincident with or shortly after starting other anti-viral therapy were excluded), 14 gained weight, 3 experienced weight loss, and 9 remained stable. There was no correlation between weight change and dosage of hCG (data not shown). There was however a pattern observed in some patients where an initial weight gain was followed by a return to baseline levels
10 while others experienced sustained weight gain over several months.

hCG therapy was well tolerated clinically by patients and there was no evidence for an adverse effect of hCG on viral load or CD4$^+$ T cell level. In two cases with advanced HIV disease hCG was discontinued because of coincident cholestasis probably due to other medications in one case and opportunistic
15 infections in the other.

Certain preparations of hCG and β-hCG were particularly efficacious in reversing wasting associated with HIV or SIV infection although variability among different preparations was observed. The native hCG and native β-chain preparations available for clinical use are not homogenous and may be
20 contaminated with one or more other active molecules. In this respect, it is noteworthy that though the effects of some preparations of hCG described here were obtained with two different commercial sources of hCG (APL and Pregnyl), one was usually more active (APL) at lower concentrations than any other preparation despite the fact that identical amounts (International Units) were
25 used, as assessed by the manufacturer's standard bioassays for the conventional use of hCG. The differences in activities of commercial preparations might be explained by variation in the amount of β-hCG peptide fragments. This could be the consequence of different methods of preparation or different sources of

human urine. For example, free β chain is more abundant in the earliest weeks of pregnancy.

6.5   EFFECTS OF hCG PREPARATIONS ON HIV-1 INFECTION *IN VITRO*

To prepare HIV infected cells for the in vitro assay of hCG preparations and β-
5   hCG peptides and derivatives, primary PBMCs, macrophages, and $CD4^+$ T cells isolated from peripheral blood and the H9 human T cell line, were acutely or chronically infected with 8 different HIV-1 strains: 4 cell line adapted viruses, namely the macrophage tropic Ba-L strain (Gartner et al., 1986, *Science* 233:215-219) and the $CD4^+$ T cell tropic MN, RF, and IIIB strains (10^5
10   $TCID_{50}$/ml) (Popovic et al., 1984, *Science* 204:497-500; Gallo et al., 1984, *Science* 224:500-503); 2 isolates, Ju1083 and G3, from Nigerian AIDS patients passed once in a $CD4^+$ T cell line (Sub-T1); and 2 primary ("clinical") isolates from AIDS patients from Trinidad which were never passed in any cell line and were used at a titer of 7.5 x 10^4 $TCID_{50}$/ml. In all experiments, HIV-1 (10^5
15   $TCID_{50}$/ml) was added to the cells (10^6 cells/ml) for a 2 hour incubation after which the virus infected cells were washed with 10 ml phosphate buffered saline (PBS) three times to eliminate extracellular virus. The test inhibitor, *i.e.* the hCG or β-hCG peptide preparation was then added and the cells were incubated with the test inhibiter for 9 to 10 days with serial sampling. Cultures
20   were assayed for p24 antigen on days 3 to 10. The inhibition of HIV production by the active preparations was not due to cell toxicity since, at the concentrations used, there was little or no effect on ^{3}HTdR incorporation, or cell viability as assessed by cell counts and 3-[4,5 dimethylthiazol-2-y], 2,5 diphenyltetrazolium, and bromide thiazoylblue (MTT) assays (data not shown).

25   There were no significant differences in the results with the different strains of HIV-1 (not shown). For illustrative purposes, the data presented are from the assays with the HIV-1 IIIB infected isolated $CD4^+$ T cells (Figure 4A), IIIB infected PBMCs (Figure 4B) and HIV-1 Ba-L infected isolated macrophages

(Figures 4C and D). As shown in Figures 4A-D, the inhibitory effects of the peptides or hCG preparations were approximately the same for macrophage tropic (Figures 4C and D) or T cell tropic strains (Figures 4A and B). Infection with primary isolates showed similar inhibition (data not shown). In contrast

5   to the potent inhibition of acute HIV-1 infection *in vitro* by the active hCG preparations or synthetic peptides, there was slight or moderate inhibition (20-40%) of virus production (HIV-1 IIIB) from chronically infected CD4$^+$ T cell lines (not shown). All target cells had similar patterns of inhibition with the expected variation in p24 antigen expression (Figures 4A-D).

10   As already noted, there is significant variation in the activity of various commercial preparations of native hCG to kill KS tumor cells (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med* 335:1261-1269). As shown in Figure 4D, there is similar variation in the anti-HIV activities of these

15   preparations. For example, for the native heterodimer preparations, the most active preparation was usually hCG APL™ (Wyeth Ayerst) and Sigma hCG (CG10) followed by hCG PREGNYL™ (Organon) (Figure 4D and Table 3).

Employing APL™ hCG, there is a dose dependent inhibition of HIV-1 replication (Figure 4A). Surprisingly, there was little or no inhibition with the highly

20   purified native hCG heterodimer preparations CR1XY17B (data not shown) and CR127 (Figures 4C and D) (generously supplied by the National Hormone and Pituitary Program and Center for Population Research, NIH) nor with purified recombinant α- or β-chains (Sigma). However, commercially available hCG (APL™, Wyeth Ayerst) and partially purified native β-hCG (NβhCG) (dissociated

25   from hCG, Sigma) was active while native α-hCG was not (Figures 4A-D and Table 3).

153

6.6   EFFECT OF hCG PREPARATIONS ON HIV-1 TRANSCRIPTION

To further elucidate the anti-HIV effects of crude hCG preparations, we studied the effect of APL™ hCG on a HIV-1 LTR driven expression of a reporter gene, chloramphenicol acetyltransferase (CAT) (Figures 5A-C). The T-lymphocyte cell

5   line HUT 78 was transiently transfected with the HIV-LTR construct 174WTIICAT by electroporation. 1×10^7 cells were resuspended in 0.4 ml RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), and 20 µg of the test plasmid with 2 µg of the Tat expression vector pDEX/Tat were introduced into the cells by a pulse from a Biorad GenePulser II apparatus of 250 V and 950 µF

10   at 4°C. (Plasmids were the generous gift of Dr. Richard Gaynor, University of Texas Southwestern Medical Center.) Cells were then divided into three aliquots and maintained at 37°C, 5% CO_2 for 40 hours in the presence of drug, or an equal volume of diluent. Transiently transfected cells were harvested, lysed and a standard amount (4 µg) of heat-treated extract was incubated in the

15   presence of 0.6 mM acetyl coenzyme A and 0.1 µCi [^{14}C]chloramphenicol in 0.25 mM Tris, pH 7.9 at 37°C for 1 hour. The amount of acetylated [^{14}C] chloramphenicol converted to acetyl [^{14}C] chloramphenicol was determined by thin layer chromatography in chloroform: methanol 95:5 (v/v) to fractionate the reaction mixture. Results were quantified by phosphorimage analysis on a

20   Molecular Dynamics Phosphor Imager 445 SI. For each assay the amount of acetylated chloramphenicol was determined as a fraction of total [^{14}C] in the sample to determine the activity of the CAT enzyme.

The expression of CAT driven by the HIV-1 LTR was inhibited in a dose-dependent manner such that 78% of normal transcription from the LTR was

25   detected in cells treated with 100 IU/ml hCG (APL™) and 36% of normal transcription was detected in cells treated with 500 IU/ml hCG (APL™) (Figure 5A). hCG had no effect on transcription of the SV-40 driven CAT construct (Figure 5B). The hCG preparation also had no inhibitory or cytotoxic effect on these cells even after 40-hour incubation with 500 IU/ml hCG (Figure 5C), as

30   previously shown (Lunardi-Iskandar, Y., et al., 1995, Nature 375:64-68).

Results from constructs with point mutations in the enhancer (NFkB), SP-1 and TAR regions of the promoter showed essentially the same response to hCG; therefore, none of these important regulatory elements was demonstrated to be necessary for the response to hCG (not shown). These results are in accord with

5 the transgenic mice results and indicate that at least part of the inhibitory effect of these hCG preparations is on transcription of the HIV-1 provirus.

6.7 EFFECTS OF β-hCG PEPTIDES ON HIV-1 INFECTION

Since it is known that hCG has proteolytic products which may co-purify with the heterodimer or its β-chain, a series of α- and β-subunits and various

10 synthetic peptides were compared to the active preparations of the native heterodimer (Figures 4A-D; see also Table 3). Peptides of the α- and β-subunits (0.05 to 50 nmoles/ml), native α chain preparation, and various crude preparations of the native hCG heterodimer (0.01 to 1,000 IU) and the native β-hCG subunit (100 to 1,000 μg) were tested for effects on HIV-1 replication in

15 acutely infected cells by measuring change in viral antigen levels in response to the above noted preparations (Figures 4A-D and Table 3).

The effect of β-hCG synthetic peptides β-hCG 45-57 (SEQ. ID NO:6; "satellin A1"), circularized β-hCG 45-57 with cysteine at position 44 (SEQ ID NO:26; "satellin A2"), the peptide β-hCG 109-119 (SEQ ID NO:7; "satellin B"), and the

20 fused peptides of amino acids 47-57 (SEQ ID NO:28) linked by a peptide bond to the N-terminus of 108-119 (SEQ ID NO:29), and 45-57 (SEQ ID No:6) linked at C-terminus by a peptide bond to the N-terminus of 109-119 (SEQ ID NO:7) all inhibit HIV-1 replication in vitro (Table 3). The first three of these peptides were shown to inhibit HIV infection in a dose dependent fashion (Figures 4A,

25 B and C) and had comparable activity in various cell systems (Figures 4A, B and C) and against various viral strains (not shown), including primary field isolates. Additionally, the β-hCG peptides of amino acids 109-145, 46-65, 7-40, 47-55 and 48-56 (SEQ ID NOS:25, 34, 33, 20 and 35, respectively) also inhibited HIV-

155

1 replication (Table 3).   Surprisingly, the "Scrambled Satellin A1", with a sequence of Cys-Val-Gln-Pro-Gly-Pro-Gly-Val-Leu-Leu-Val-Leu-Cys (SEQ ID NO: 36) also had an inhibitory effect (Table 3). Native α-hCG, the α-hCG peptides of 88-92 and 1-95, and several other β-hCG peptides had little or no effect

5   (Figures 4A, B and C and Table 3).

6.8   EFFECTS OF β-hCG PEPTIDES ON KAPOSI SARCOMA CELLS

Neoplastic Kaposi's Sarcoma tumor cells with a characteristic chromosomal abnormality have been reported (Delli-Bovi et al., 1986, *Cancer Res.* 46:6333-6338; Siegal, et al., 1990, *Cancer* 65:492-498; Popescu et al., 1995,

10   *JNCI* 88:450-454) and provide a model system for studying the *in vitro* effects of hCG on KS cells. In our prior studies employing immune deficient mice injected with KS tumor cells, some commercial preparations of native hCG killed KS tumor cells *in vivo* apparently by inducing apoptosis and inhibiting angiogenesis. *In vitro* tumor cell colonies were also suppressed in clonogenic

15   assays by the hCG preparations (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68; Nakamura et al., 1988, *Science* 242:426-430; Ensoli et al., 1989, *Science* 243:223-226; Salahuddin et al., 1988, *Science* 242:430-433; Masood, et al., 1984, *AIDS Res. Hum. Retroviruses* 10:969-976). In the current study, experiments were performed to investigate whether the anti-viral effect of the

20   active peptides (Figures 4A-D and Table 3) correlated with the anti-KS effect of native hCG both *in vitro* in clonogenic assays on cultured KS Y-1 cells and *in vivo* in KS tumors induced in nude mice by injection of cultured Kaposi's Sarcoma cells.

Briefly, the KS Y-1 cells were obtained from mononuclear cells isolated from

25   pleural effusion of an AIDS patient with KS in the lungs. After the depletion of T lymphocytes, monocytes/macrophages and fibroblasts using monoclonal antibodies against CD2, CD3, CD4, CD8, CD10 and CD14 membrane antigens and baby rabbit complement, the cells were cultured in the absence of

exogenous growth factors to select for transformed cells. Immunological characterization of the KS Y-1 cells showed that CD34, CD31 and endoglin were expressed. Clonogenic assays were performed by seeding the KS Y-1 or KS-SLK cells in methylcellulose (0.8%, v/v), incubating the cells for 10 days in the
5   presence or absence of the hCG, β-hCG or β-hCG peptide preparations and then counting the number of well-formed colonies of triplicate wells formed after seeding with 5 X 10⁴ cells.

As shown in Figure 6A and Table 3, the peptides used in the assay at a concentration of (50 nmoles/ml) with the strongest anti-viral effects (peptides
10   of amino acids 45-57 (SEQ ID NO:6), cyclic 44-57, with cysteine substituted at position 44 (SEQ ID NO:26), 109-119 (SEQ ID NO:7), 109-145 (SEQ ID NO:25), and 47-57 linked at the C-terminus by a peptide bond to the N-terminus of 108-119 (SEQ ID NO:32), and 45-57 linked at the C-terminus by a peptide bond to the N-terminus of 109-119 (SEQ ID NO:30)) also had the strongest anti-tumor
15   effects (i.e., anti-KS effect) on the two KS neoplastic cell lines. It is notable that the highly purified hCG heterodimer (CR127 2 use data concentrations of nmoles/ml) was inactive, as in the *in vitro* HIV assay. There was no anti-KS effect with the highly purified α- and β-chains and the α-hCG peptides, and other β-hCG peptides showed little or no inhibition in clonogenic assays (Table
20   3). Again, the "Scrambled Satellin A1" peptide (SEQ ID NO:36) exhibited activity while the others scrambled peptides did not.

The effects of the peptides on KS tumor cells were also evaluated *in vivo* in the KS mouse model. To induce KS tumors in the mice, 1 x 10⁶/ml KS Y-1 cells in 50 μl PBS or saline were injected subcutaneously into immunodeficient mice
25   (beige-XID-BNX mice). After one week, tumors ranged in size from 2 mm x 3 mm to 3 mm x 5 mm. Methods for detection of apoptosis (from tissue biopsies) were used, as described in Lunardi-Iskandar et al.(1995, *Nature* 375:64-68). Briefly, the samples were stained *in situ* for the presence of cells with DNA fragmentation. Tissue slides from formalin-fixed tumors were treated with

terminal deoxynucleotide transferase for extension of DNA ends (hydroxyl 3')
and incorporation of digoxigenin-11-dUTP according to the manufacturer's
instructions (Oncor, Gaithersburg, MD). Anti-digoxigenin antibody conjugated
with the enzyme peroxidase allowed detection of apoptotic cells, which stain

5   brown, whereas viable cells stain blue.

Shown in Figures 6B-E are representative examples of the effects of hCG and the
β-hCG peptides on KS Y-1 tumors in mice. One week after injection with the
tumor cells, the mice were treated with crude hCG (APL™, Wyeth Ayerst) or
with β-chain peptides 45-57 (SEQ ID NO:6) and cyclic 44-57[Cys44] (SEQ ID

10   NO:26). Figures 6B-E show hematoxylin and eosin staining of thin tissue
sections of KS Y-1 induced tumors. Compared to the frequent mitotic activity
in the controls (Figure 6B), there is evidence of extensive cell death in the
tumors of the animals treated with the β-hCG peptides which are comparable to
the findings in animals treated with active hCG preparations (Figures 6C-E).

15   Table 3 presents data showing that the β-hCG peptides with an amino acid
sequence of 47-57 linked at the C-terminus by a peptide bond to the N-terminus
of 108-119 (47-57::108-119; SEQ ID NO:32) and 45-57 linked at the C-terminus
by a peptide bond to the N-terminus of 109-119 (45-57::109-119; SEQ ID
NO:30) also had significant anti-KS activity. Additionally, β-hCG peptides of

20   amino acid numbers 109-119, 109-145, 47-55 and 48-56 (SEQ ID NOS:7, 25, 20
and 35, respectively) exhibited some anti-KS activity. Other α-hCG and β-hCG
peptides showed no activity (Table 3).

As noted above, some AIDS-KS patients treated by intralesional or systemic
injection of some preparations of hCG experience regression of tumor lesions

25   of the skin as well as visceral KS (Hermans et al., 1995, *Cellular and Molecular
Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269).
Patients receiving these preparations showed macroscopic regression and
flattening of KS lesions. In situ immunostaining specific for apoptosis detection
in tumor biopsies showed evidence of apoptosis and/or, histologically, complete

absence of the KS tumor after 2-3 weeks of hCG therapy as shown in Figures 6F, G and H, similar to that seen in the experimental mouse model with the active β-hCG peptides. In control KS tumors treated with diluent only or untreated KS tumor tissues (not shown), there was little evidence of cell death (Figure 6F).

5   ## 6.9   EFFECTS OF hCG AND β-hCG PEPTIDES ON HEMATOPOIESIS

In addition to the typical decline in CD4$^+$ T cells, cytopenias can occur in HIV infected people affecting one or more hematopoietic lineages associated with deficient progenitor cell growth. This deficiency is often made worse by some of the anti-viral therapies currently in use. In contrast, hCG preparations do not

10   inhibit hematopoiesis.

The effect of hCG preparations and peptides was assayed on hematopoietic progenitor cells in vitro. Hematopoietic progenitor cells (2 x 10^5 cell/ml) were isolated from normal bone marrow and cord blood and seeded in methylcellulose. The following hCG preparations and peptides were used in

15   these clonogenic assays were: hCG (APL™); hCG alpha subunit (Sigma); purified hCG heterodimer CR 127; β-hCG peptide 109-119 (SEQ ID NO:7) (Bachem); β-hCG peptide 45-57 (SEQ ID NO:6); β-hCG peptide 45-57 circularized (44-57 with cysteine substituted for the amino acid at position 44, SEQ ID NO:26); mixture of scrambled β-hCG peptides 45-57 and 109-119; and

20   crude preparation of native β-hCG. Also tested were the peptides 45-57 linked at the C-terminus via a peptide bond to the N-terminus of 109-119 (45-57::109-119; SEQ ID NO:30), 47-55 (SEQ ID NO:20) and 48-56 (SEQ ID NO:35) as well as other hCG, α-hCG and β-hCG preparations and α-hCG and β-hCG peptides (Table 3). The hCG preparations were administered at 200 IU/ml and the β- and

25   α- subunits and peptides were administered at 100 μg/ml. The native commercial preparation of hCG (APL™, Wyeth Ayerst) was pre-tested for

159

anti-HIV and anti-KS activities. Aggregates containing more than 50 cells after 10 days of culture were counted as colonies.

As shown in Figures 7A-C and in Table 3, the growth of hematopoietic progenitors (Lunardi-Iskandar et al., 1989, *Leukemia Res.* <u>13</u>:573-581) is

5   directly promoted by a commercial preparation of partially purified hCG (APL™, Wyeth Ayerst), partially purified native β-chain, and by the synthetic peptides, β-hCG peptide 45-57 (SEQ ID NO:6), β-hCG peptide 109-119 (SEQ ID NO:7), circularized 44-57 with cysteine substituted for the amino acid at position 44 (SEQ ID NO:26), the peptide 45-57 linked at the C-terminus via a peptide bond

10   to the N-terminus of 109-119 (45-57::109-119; SEQ ID NO:30), and a mixture of the β-hCG peptides 45-57 and 109-119 (SEQ ID NOS:6 and 7, respectively), but not by the highly purified hCG heterodimer (CR127) nor by the recombinant hCG β-chain or the α-chain preparations. The β-hCG peptides 47-55 and 48-56 (SEQ ID NOS:20 and 35, respectively) also exhibited a pro-hematopoietic effect

15   (Table 3). Additionally, scrambled β-hCG peptides 45-57 and 109-119 as well as other β-hCG peptides showed little inhibition (Table 3). Thus, these results recapitulate the anti-KS and anti-HIV effects. Each activity is chiefly effected by the satellin peptides (45-57 and 109-119; SEQ ID NOS:6 and 7, respectively). A series of other peptides of the α- and β-chain had no effect (data not shown).

20   Some partially purified preparations of hCG and β-hCG and some β-chain fragments stimulate the growth of hematopoietic progenitors, for example β-hCG peptides having amino acid sequences of amino acid numbers 45-57 (SEQ ID NO:6), 109-119 (SEQ ID NO:7), circularized 44-57, where cysteine is substituted for the amino acid at position 44 (SEQ ID NO:26), and peptides of

25   amino acid numbers 45-57 (SEQ ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or a peptide of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide bond to the N-terminus of amino acids 108-119 (SEQ ID

NO:29) of the β-hCG sequence depicted in Figure 8 (portions of SEQ ID NO:2). The peptides having an amino acid sequence of amino acid numbers 7-45, 47-55, 46-65, and 48-56 (SEQ ID NOS:21 and 33-35, respectively) of β-hCG (Figure 8 (SEQ ID NO:2) also exhibit activity in *in vitro* assays.

5   Furthermore, in 3 of 3 SIV acutely infected rhesus macaque monkeys the same preparation of hCG (pre-screened for anti viral activity) at a dose of 6,000 IU per week, led to a reduction of SIV in plasma, an increase in CD4$^+$ T cells and weight gain. Examples of pro-hematopoietic effects are observed in preliminary studies of a limited number of patients treated with some commercial hCG
10   products. Factors such as patient stage, total weekly dose, and manufacturer source very likely play a role in the variability of response.

New treatment regimens for HIV-1 show that a combination of anti-HIV compounds which target reverse transcriptase (RT) such as azidothymidine (AZT), lamivudine (3TC), dideoxyinosine (ddI), dideoxycytodine (ddC) used in
15   combination with an HIV-1 protease inhibitor, have a far greater effect (2 or more logs reduction) on viral load compared to AZT alone (about 1 log reduction) (Perelson et al., 1996, *Science* 15:1582-1586). However, long-term use of combinations of these chemicals may lead to toxicity, especially to the bone marrow and suppression of CD8$^+$ T cells, which may be essential to the
20   control of HIV via killer cell activity (Blazevic et al., 1995, *AIDS Res Hum Retroviruses* 11:1335-1342) and by the release of suppressive factors, notably the C-C chemokines (Cocchi et al., 1995, *Science* 270:1811-1815). Other concerns in long-term chemical anti-retroviral therapy are the possible development of HIV mutations with partial or complete resistance (Lange, 1995,
25   *AIDS Res Hum Retroviruses* 10:S77-82) and cost.

The discovery of an anti-KS effect of "hCG" was observed *in vivo* in pregnant Bg-nude mice which did not develop KS as did their male litter mates inoculated at the same time with the KS Y-1 tumor cells. This observation led to clinical

161

trials of intralesional therapy for KS which documented responses in 83% of treated lesions in a dose dependent manner (Hermans et al., 1995, *Cellular and Molecular Biology* 3:357-364; Gill et al., 1996, *New Engl. J. Med.* 335:1261-1269).

5   As shown herein, some patients treated intralesionally with an hCG preparation for KS were noted to have a reduction in viral load, and *in vitro* human cell culture and *in vivo* animal model data show that some preparations of partially purified hCG, partially purified β-hCG and the active β-hCG peptides and fused β-hCG peptides have anti-viral, anti-KS and pro-hematopoietic effects.

10   We found considerable activity with some preparations of the partially purified native heterodimer and the native partially purified whole β-chain, whereas recombinant β-hCG (purified) and highly purified native hCG heterodimer (CRIXY17B and CR127) were inactive. Based on our results with the β-hCG peptides and fused β-hCG peptides reported here, we suspect that the lower

15   molecular weight species, active portions of them, or possibly larger fragments which include the active amino acid sequences accompany hCG and the β-chain and are not eliminated by some of the purification procedures, thus retaining the anti-viral, anti-KS and pro-hematopoietic effects, but varying among commercial sources. In this respect, it is noteworthy that, although the clinical

20   effects of some preparations of hCG described here were obtained with two different commercial sources (APL™ and PREGNYL™), one was usually more active in laboratory tests (APL™) at lower concentrations than any other preparation. This hCG preparation, however, also varied from lot to lot in the immunodeficient mouse KS system (data not shown) despite the fact that

25   identical amounts (International Units) were used as assessed by the manufacturer's standard bioassays for the conventional use of hCG.

Consequently, we next began studies with a variety of synthetic peptides, and our results show that all the *in vitro* activities of the preparations of native hCG

and the *in vivo* mouse data are mimicked strongly by satellin A1 (β-hCG peptide 45-57 (SEQ ID NO:6)), but not other β- or α-peptides or scrambled 45-57 peptide.

5   The mechanism of the anti-HIV effect of some preparations of native hCG, and native β-hCG, and of the β fragments appear, at least in part, to be direct. This is suggested by: 1) the *in vitro* inhibition of HIV-1 infectivity of CD4$^+$ T cells and macrophages; 2) the inhibition of HIV-1 gene transcription in HIV-1 transgenic mice; 3) the rapid clearance of p27 antigen in the acutely SIV infected monkeys treated with hCG; and 4) the decline of plasma virus in some 10   patients treated with some hCG preparations. However, there was a greater inhibition of *in vitro* infection of cells with various strains of HIV-1 compared to inhibition of HIV-1 production from chronically infected cells suggesting that mechanisms, in addition to inhibition of transcription, are also involved. Indirect effects also cannot be excluded for the anti-SIV/HIV effects observed 15   in the monkey experiments and among responding patients. HIV has anti-hematopoiesis effects (Lunardi-Iskandar et al., 1989, *J. Clin. Invest.* 83:610-615; Louache et al., 1992, *Blood* 180:2991-2999; Geller et al., 1985, *Archs. Path. Lab. Met.* 109:138-145). Based on the findings that hCG and the peptides have pro-hematopoietic effects on progenitors cells of the bone 20   marrow, it is possible that enhanced immune function also may have contributed to the *in vivo* results.

Some preparations of hCG have beneficial effects against the range of core problems associated with HIV-1 infection. In laboratory tests, KS cells were killed and regression occurred of transplanted KS tumors in mice 25   (Lunardi-Iskandar et al., 1995, *Nature* 375:64-68). A recent clinical study of escalating dose by intralesional injection of hCG (APL™, Wyeth Ayerst) for cutaneous KS skin lesions demonstrated tumor regression in a dose-dependent manner, with 8% responding at the lowest dose (250 IU, 3 times weekly) and 83% at the highest intralesional dose (2000 IU, 3 times weekly) (Gill et al.,

1996, *New Engl. J. Med.* <u>335</u>:1261-1269). Results described here also showed regression of KS lesions in a substantial proportion of cases including cases treated with systemic therapy, and even regression of newly developed lesions while on hCG therapy when higher doses were given. It is also noteworthy that
5   regression of visceral lesions occurred in several KS patients with advanced KS.

The clinical data reviewed herein illustrate many of the beneficial effects observed in the laboratory pre-clinical studies. Since the protocols were not designed to systematically study the various beneficial effects of some preparations of hCG as a treatment for HIV infection and since there is
10   variability in dose and source of product, the inferences to be drawn are illustrative of the potential for some commercial preparations of hCG or related products in HIV and KS treatment. As reported elsewhere (Hermans et al., 1995, *Cellular and Molecular Biology* <u>3</u>:357-364; Gill et al., 1996, *New Engl. J. Med.* <u>335</u>:1261-1269), and confirmed herein, some preparations of hCG induced partial or complete regression of KS lesions in patients treated
15   intralesionally (Hermans et al., 1995, *Cellular and Molecular Biology* <u>3</u>:357-364) and systemically, including advanced visceral disease. In some HIV-1 positive patients at various stages of HIV infection there was a 0.5 to 2 log reduction in plasma viremia level, and in some cases this effect was sustained with no evidence of development of resistance or toxicity and $CD4^+$
20   T cell levels increased in some as well. Non-fluid weight gain was a very frequent benefit to patients even with far advanced disease. It is noteworthy that patients such as PH-VE have experienced long term benefits from hCG therapy without toxicity over more than 80 weeks of therapy documenting the
25   safety of this therapeutic approach.

6.10   FRACTIONATION OF ACTIVE hCG PREPARATIONS AND HUMAN EARLY PREGNANCY URINE

The present inventors have found that certain commercial preparations of hCG, for example, hCG APL™ (Wyeth-Ayerst), had higher anti-HIV, anti-Kaposi's

164

Sarcoma, anti-wasting and pro-hematopoietic activity than other commercial preparations of hCG (see discussion herein). Further, the inventors have also shown that highly purified preparations of native and recombinant hCG and β-hCG had no activity against HIV infection or replication or against Kaposi's

5 Sarcoma. Accordingly, the inventors postulated that there must be an activity in the hCG commercial preparations that is not the hCG dimer or the β-hCG subunit, responsible for the anti-HIV, anti-Kaposi's Sarcoma, anti-wasting and pro-hematopoietic activities. This section presents results of the fractionation of the APL™ hCG commercial preparation and urine from women in the first

10 trimester of pregnancy ("human early pregnancy urine") which also contains hCG. Particular sizing column chromatography fractions were shown to have activity, thus demonstrating that the active components could be fractionated.

6.10.1 MATERIALS AND METHODS

Both human early pregnancy urine and the APL™ (Wyeth-Ayerst) hCG

15 commercial preparation were subjected to fractionation. For the human early pregnancy urine, 5 liters of urine were collected from women in the first trimester of pregnancy. Twenty-four hour collections were stored frozen or refrigerated for up to 2 days. Upon delivery of the urine to the laboratory, sodium azide was added at 1 g/liter and the urine frozen until five liters had

20 been collected. At this time, all the urine was thawed overnight, and the pH was adjusted to 7.2-7.4 with NaOH, which causes some precipitation. The precipitate was allowed to sediment for 1 hour at room temperature, most of the supernatant decanted and the remaining supernatant centrifuged to remove any additional precipitate with that supernatant being added to the first supernatant

25 decanted. Next, the urine was concentrated with a Pellicon (Millipore) filtration system using a membrane cassette with a 3,000 MW cut off, which concentrates the urine approximately 60 to 80 fold. Next, the urine was desalted and delipidated by passing 500 ml of the material at a time through a Sephadex G25 column with a volume of 1.7 liters in 0.05 M ammonium

165

bicarbonate (the column was washed between runs with 25% ethanol to remove absorbed lipids and glycoprotein). The material was lyophilized and stored for further fractionation. The urinary material was then reconstituted in 6 ml of 30 mM sodium phosphate buffer, pH 8.3 fractionation.

5   For the APL™ hCG, the lyophilized hCG preparation from eleven vials (each vial containing 20,000 IU hCG) was resuspended in 6 ml of 30 mM sodium phosphate buffer, pH 8.3 and filtered twice through the 0.45 µm particle filter. For both the lyophilized urine and the hCG APL™, the prepared sample was then loaded onto a pre-packed SUPERDEX™ 200 HiLoad Column (Pharmacia 26

10   mm² x 60 cm) in the 30 mM sodium phosphate buffer, pH 8.3 and then eluted from the column with a solution containing 30 mM sodium phosphate buffer, pH 7.0 and 2 M NaCl. For the first ten minutes, the column flow rate was 1 ml/minute (due to the viscosity of the hCG APL™ material; this flow rate scheme was also used for the urine material); after the first 10 minutes, the flow rate

15   was 2 ml/minute. The column was run on a Hewlett Packard 1050 HPLC equipped with a photodiode array detector. Four ml fractions were collected and frozen until further analysis.

The protein concentration in each fraction was determined by amino acid analysis. A 50 µl aliquot of alternate column fractions was processed for anlysis

20   by hydrolysis in vapors of 6N HCl with 0.1% phenol at 110°C for 24 hours in a Waters Associates Pico-Tag Workstation (Waters, Milford, MA). An internal standard, norleucine, was added to all fraction samples before hydrolysis to correct for any losses during hydrolysis or liquid transfer. The hydrolyzed samples were then analyzed on a Beckman Instruments 6300 amino acid

25   analyzer and the data was collected on the PE Nelson Data System (Perkin-Elmer) and transformed using PE Nelson Turbochrome software.

The column fractions were monitored with immunoassays to heterodimeric hCG as well as to the hCG beta core fragment (O'Connor et al., 1994, *Endocrin. Rev*

15:650-683; Krichevsky et al., 1994, *Endocrinology* 134:1139-145; Krichevsky et al., 1991, *Endocrinology* 128:1255-1264; O'Connor et al., 1988, *Cancer Res.* 48:1361-1366; Krichevsky, 1988, *Endocrinology* 128:584-593). These two assays permit placement of two internal standard sizes for the gel filtration

5   column: 70,000 kD (hCG) and 10,000 kD (hCG beta core fragment which is amino acids 6-40 of β-hCG linked via a disulfide bond to amino acids 55-92 of β-hCG). External molecular weight standards were also employed to calibrate the column elution positions. In addition, MALDI-TOF mass spectrometry was used to evaluate the ions observed in certain active fractions. Mass

10 spectrometry did indicate that some peptides separated at anomalous positions, showing that they were being carried by other proteins to earlier elution positions in some cases, or interacting with the column matrix and eluting much later than their molecular size would indicate. For example, 3,000 and 6,000 molecular weight materials eluted from the gel filtration column with material

15 of 14,000 molecular weight while 11,000 molecular weight material eluted with material of approximately 1,000-2,000 molecular weight, hCG and hCG-related molecules eluted at their expected positions.

The fractions were then tested for anti-HIV, anti-KS and pro-hematopoietic activities in vitro. To assay for inhibition of HIV-1 replication in vitro, the

20 HIV-1 IIID viral strain was used to infect PBMCs and PM-1 cells (derived from the HUT-78 T-cell lymphoma cell line) at 10^3 TCID$_{50}$/ml as described in detail herein. The infected cells were incubated for three days in 100 IU/ml of the hCG APL™ or β-hCG C-Sigma preparations; 50-100 µl per ml of the hCG APL™ or early pregnancy urine fractions; 50 µg/ml β-core protein or α-hCG

25 preparation; 200 IU/ml of the highly purified CR127 hCG preparation; or 100 µl/ml of the circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26).

To assay for activity against Kaposi's sarcoma cell growth in vitro, the clonogenic assay described herein was used with the KS Y-1 and KS-SLK

cultured Kaposi's Sarcoma cell lines. The cells were incubated in 200 IU/ml of commercial hCG preparations; 50 µl/ml of certain fractions from the hCG preparation of early pregnancy urine fractionation; or 100 µg/ml β- and α-hCG chains, β-hCG core protein, β-hCG peptides or LH (leuteinizing hormone).

5   Pro-hematopoietic activity was assayed in in-vitro clonogenic assays as described herein. Cells were assayed for colony formation in the presence of 200 IU/ml hCG APL™ or highly purified hCG preparation CR 127; 100 µl/ml of the fractions of the hCG commercial preparation of early pregnancy urine; or 100 µg/ml β-hCG core protein or cyclized β-hCG peptide of amino acids 44-57
10   (with cysteine substituted at position 44; SEQ ID NO:26).

Certain fractions were tested for activity in reducing Kaposi's Sarcoma lesions in the Kaposi's Sarcoma mouse model as described herein. In this assay, starting one week after injection of the KS Y-1 cells to induce Kaposi's sarcoma formation, the mice were injected subcutaneously with 100 IU hCG APL™; 200
15   IU highly purified hCG preparation CR127; 100 µg α-hCG, β-hCG, recombinant β-hCG, LH (luteinizing hormone), or β-core protein; 200 µl of fractions of commercial hCG preparation or early pregnancy urine; or 200 µg cyclized β-hCG peptide of amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) per day for one week. After one week of the week of treatment with
20   the hCG fractions, the KS lesions were examined for cell apoptosis and regression as described herein.

Finally, certain fractions were tested for their ability to increase survival, promote weight gain and reduce HIV-1 gene expression in HIV-1 transgenic mice as described herein. The mothers the mice were administered 300 IU hCG
25   APL™ by osmotic pump or 200 IU hCG APL™ by slow release; 200 µl of certain fractions of hCG commercial preparation or of early pregnancy urine; 200 µg of the cyclized β-hCG peptide of amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) or the fused peptide of amino acids 45-57::109-

119 (SEQ ID NO:30); or 100 μg β-hCG core peptide or the α-hCG sub unit per day, and the pups were dosed through the mother's milk.

The unfractionated APL™ hCG preparation, PREGNYL™ (Organon) hCG preparation, purified β-core and phenol were also tested in certain assays.

5 Phenol, which is an additive in the hCG APL™ preparation, was tested to control for any effect on cell growth or viral inhibition.

6.10.2   ACTIVE FRACTIONS

Fractionation of both the APL™ hCG preparation and the human early pregnancy urine resulted in a significant protein peak at approximately 158 kD with

10 diminishing, but still detectable, protein in the rest of the fractions, even those containing small molecular weight material (Figures 10A and D). Fractions containing the hCG dimer (77 kD) and the β-hCG core (10 kD) were identified by immunoprecipitation using antibodies that specifically recognize these particular species, as described in the materials and methods herein. The

15 elution profile of the commercial hCG material was also plotted in comparison to the elution of standard molecular weight markers (Figures 16A and B). Additionally, Fractions 61, 63, 64, 65 and 67 from the fractionation of the commercial hCG material was analyzed by MALDI-TOF mass spectrometry (Figures 17A-E, respectively).

20 ### 6.10.3   EFFECT OF FRACTIONS OF COMMERCIAL hCG PREPARATIONS AND EARLY PREGNANCY URINE ON HIV-1 REPLICATION IN VITRO

The fractions of both the APL™ hCG preparation and the human early pregnancy urine were assayed for inhibition of HIV-1 IIID replication in PBMCs and PM-1 cells as described herein. Many of the APL™ hCG preparation fractions

25 exhibited significant inhibition of HIV-1 IIID replication (Figure 10C). In particular, fractions containing material of approximately 70 kD to approximately 2-3 kD exhibited HIV-1 inhibitory activity. The fractions

effecting the highest percent inhibition of HIV-1 replication were fractions 62, 63, 65, and 73, with the three main peaks of activity eluting with apparent molecular weights of approximately 40 kD, approximately 15 kD, and approximately 2-3 kD, as determined by comparison with the elution of hCG
5   (77 kD) and β-core protein (10 kD).

The fractions of human early pregnancy urine were also assayed for ability to inhibit HIV-1 IIID replication in the PBMCs and the PM-1 cells. Again, several fractions had at least some HIV-1 replication-inhibitory activity. Fractions 64 and 67 caused more than twice the inhibition of HIV-1 IIID replication than any
10   of the other fractions (Figure 10F). There were approximately two peaks of activity eluting from the gel filtration column with apparent molecular weights of approximately 15 kD and 3 kD, as determined by comparison with the elution of hCG (77 D) and β-core protein (10 kD) identified by immunoassay.

Additionally, phenol had no effect on HIV-1 replication, demonstrating that the anti-HIV activity of the APL™ hCG is not due to the presence of phenol in the
15   APL™ hCG preparation, and purified β-hCG core protein (the peptide of amino acids to 6-40 of β-hCG linked via a disulfide bond to the peptide of amino acids 55-92 of β-hCG as depicted in Figure 8 (SEQ ID NO:2)) was also found not to inhibit HIV-1 replication (data not shown).

20   **6.10.4   EFFECT OF FRACTIONS OF COMMERCIAL hCG AND EARLY PREGNANCY URINE ON KAPOSI'S SARCOMA CELL GROWTH IN VITRO**

The fractions of APL™ hCG and human early pregnancy urine were also tested for inhibition of the proliferation of cultured Kaposi's Sarcoma cells. Figure
25   10B depicts the results of assays of the APL™ hCG fractions for inhibition of KS Y-1 cell growth. There were three major peaks of KS cell growth inhibitory activity which eluted from the gel filtration column with apparent molecular weights of approximately 40 kD, approximately 15 kD, and approximately 2-3

kD, as compared with the elutions of fractions containing hCG dimer (77 kD) and β-core protein (10 kD). A fraction containing material about the same size as the β-hCG core protein exhibited the highest level of inhibition; however, purified β-hCG core was found not to inhibit KS cell growth (data not shown).

5 Fractions of human early pregnancy urine were also assayed for inhibition of KS Y-1 cell growth. Fractions containing material which eluted from the gel filtration column with apparent molecular weights of approximately 15 kD and approximately 2-3 kD as compared with the elution of fractions containing hCG dimer (77 kD) and the β-hCG core (10 kD) as identified by immunoprecipitation
10 assay were the most effective at inhibiting KS cell growth, with the approximately 15 kD fractions having the highest activity (Figure 10E).

Figure 11 presents additional data on the inhibitory effects of hCG and hCG-related preparations in KS cultured cell clonogenic assays using both the KS Y-1 and KS SLK assays. Fraction 65 (from the peak eluting with an apparent molecular weight of approximately 15 kD) and 76 (from the peak eluting with
15 an apparent molecular weight of approximately 2-3 kD) from the fractionation of both the APL™ hCG preparation (fraction 65 and 76 are represented by bars 12 and 13, respectively,) and the early pregnancy urine (fraction 65 and 76 are represented by bars 10 and 11, respectively) inhibited growth of both cell lines.
20 The fractions containing material eluting with an apparent molecular weight of approximately 2-3 kD (i.e. fraction 76 of both fractionations) inhibited KS cell growth marginally more effectively than the fractions containing material eluting with an apparent molecular weight of approximately 15 kD (i.e. fraction 65 of both fractionations). Although the active fractions elute close to the
25 fractions containing the β-hCG core protein, purified β-hCG core protein (bar 5) exhibited almost no inhibition of KS cell growth.

The results confirm that the APL™ hCG commercial preparation (bar 1) inhibited KS cell growth better than the other commercial hCG preparations (bars 2-4).

171

Additionally, while native β-hCG (bar 6) inhibited KS cell growth moderately well, α-hCG, the highly purified hCG preparation CR 127 and recombinant hCG (Sigma) (bars 7-9, respectively) inhibit the KS cell growth negligibly. The results also confirm that the cyclized β-hCG peptide of amino acids 44-57

5 (cysteine substituted at position 44; SEQ ID NO: 26) also inhibited KS cell growth.

6.10.5 EFFECT OF COMMERCIAL hCG AND EARLY PREGNANCY URINE FRACTIONS ON HEMATOPOIESIS IN VIVO

10 Figures 12A-C presents data on the effect of hCG and hCG-related preparations on hematopoiesis in in vitro clonogenic assays for numbers of colony forming units of granulocytes, erythrocytes, megakaryocytes and monocytes (CFU-GEMM), burst forming units of erythrocytes (BFU-e) and colony forming units of granulocytes and macrophages (CFU-GM). Figure 12 shows that fraction 65

15 of both the hCG APL™ and early pregnancy urine fractionation (bars 7 and 8, respectively; fraction 65 contains material with an apparent molecular weight of approximately 15 kD) promoted hematopoiesis in all three assays. Fraction 26 of the early pregnancy urine fractionation (bar 9) did not promote hematopoiesis in any of the assays. The purified β-core protein (bar 10)

20 likewise exhibited no stimulation of hematopoiesis.

These results also confirm that the hCG APL™ preparation, native β-hCG and the cyclized β-hCG peptide of amino acids 44-57 (cysteine substituted at position 44; SEQ ID NO: 26) (bars 3, 5 and 6, respectively) all have pro-hematopoietic activity. The α-subunit of hCG, highly purified hCG preparation CR127 and PBS

25 alone (bars 2, 4 and 1, respectively) did not promote hematopoiesis.

6.10.6 EFFECTS OF hCG AND β-hCG PREPARATIONS AND β-hCG PEPTIDES ON KAPOSI'S SARCOMA

As described herein, we have observed beneficial effects of some preparations
of human Chorionic Gonadotropin (hCG) against HIV disease including
anti-tumor (Kaposi sarcoma, KS), anti-viral, increase in weight and
pro-hematopoiesis effects. Our studies document that the same preparations
inhibit KS cell growth *in vitro* and induce apoptosis in a mouse model.
Examples of these effects were also noted in some HIV-positive patients treated
with some hCG preparations. The strength of these effects varied among crude
hCG preparations, and highly purified hCG did not retain these activities.
However, the anti-KS, anti-viral, and pro-hematopoietic effects were mimicked
by native β-hCG and synthetic peptides of the beta subunit of hCG having amino
acid sequences of amino acid numbers 45-57 (SEQ ID NO:6), 109-119 (SEQ ID
NO:7), circularized 44-57, where cysteine is substituted for the amino acid at
position 44 (SEQ ID NO:26), and peptides of amino acid numbers 45-57 (SEQ
ID NO:6) linked at the C-terminus via a peptide bond to the N-terminus of
amino acids 109-119 (SEQ ID NO:7) or linked at the N-terminus via a peptide
bond to the C-terminus of amino acids 110-119 (SEQ ID NO:27); or a peptide
of amino acids 47-57 (SEQ ID NO:28) linked at the C-terminus via a peptide
bond to the N-terminus of amino acids 108-119 (SEQ ID NO:29) of the β-hCG
sequence depicted in Figure 8 (portions of SEQ ID NO:2). The peptides having
an amino acid sequence of amino acid numbers 7-45, 47-55, 46-65, and 48-56
(SEQ ID NOS:21 and 33-35, respectively) of β-hCG (Figure 8 (SEQ ID NO:2) also
exhibit activity in *in vitro* assays.

The discovery of an anti-KS effect of the pregnancy hormone, hCG, was
observed *in vivo* in pregnant Bg-nude mice who did not develop KS as did their
male litter mates inoculated at the same time with the KS Y-1 KS tumor line.
This observation led to clinical trials of intralesional therapy for KS which
documented responses in 83% of treated lesions at the higher dose schedule

173

(Gill, P.S., et al., 1996, submitted). We show herein that some patients treated intralesionally with hCG for KS were noted to have a reduction in viral load and *in vitro* and *in vivo* animal model data show that some hCG preparations, partially purified β-hCG, and the active β-hCG fragments (β-hCG peptides 45-57

5   and 109-119) have anti-KS effects.

We found considerable anti-KS activity with the native partially purified whole β-chain, but recombinant β-hCG (purified) had little or no effect. We suspect that the lower molecular weight species may retain the effect and that some purification procedure may not eliminate those species.

10 The native hCG and native β-chain preparations available for clinical use are not homogenous and may be contaminated with one or more other active molecules. In this respect, it is noteworthy that though the effects of some preparations· of hCG described here were obtained with two different commercial sources of hCG (APL and Pregnyl), one·was usually more active

15 (APL) at lower concentrations than any other preparation, although it too varied from lot to lot as detected in the immunodeficient mouse KS system (data not shown) despite the fact that we used identical amounts (International Units) as assessed by the manufacturer's standard bioassays for the conventional use of hCG.   The differences in activities of commercial preparations might be

20 explained by variation in the amount of β-hCG fragments.   This could be the consequence of different methods of preparation or different sources of human urine.   For example, free β-hCG is more abundant in the earliest weeks of pregnancy.   Consequently, we initiated studies with a variety of synthetic peptides, and our results show that all the *in vitro* activities of the preparations

25 of native hCG are mimicked by the β-hCG peptides 45-57, and 109-119 but not other β- or α-peptides or scrambled 45-57 peptide. Thus, we suggest that β-hCG contains structural motifs that produce effects which probably work by mechanisms which differ from those currently known for hCG. We suspect that β-hCG fragments have biological functions quite distinct from the conventional

174

effects of the heterodimer. The structural features of hCG (Lapthorn, A.J., et al., 1994, *Nature* 369:455-461) and appearance in very early pregnancy (Fan, C., et al., 1987, *J. Clin. Endo. Metab.* 64:313-318) combined with some of our observed effects of the β-chain peptides on Kaposi's Sarcoma tumors involving

5   induction of apoptosis (Lunardi-Iskandar, Y., et al., 1995, *Nature* 375:64-68), suggest that the structural similarity to some growth factors may be important and might also be relevant to the hematopoietic growth promoting and anti-viral effects observed here. In view of the evidence that the α subunits are needed for binding to the hCG receptor, we are uncertain how the β peptides initiate

10   these effects. Thus, whether the effects we have observed (anti-viral, anti-tumor, anti-wasting and pro-hematopoietic) are mediated by known hCG receptors is unknown. Given that the mechanism of action of these hCG fragments is likely to involve pathways distinctive from normal hCG hormonal pathways, it is proposed that these active peptides represent a new class of

15   active molecules which we named Satellins. The first members of this class are Satellin A for the active moiety from the β-hCG peptide 45-57 and Satellin B for the β-hCG peptide 109-119.

In laboratory tests, KS cells were killed and regression occurred of transplanted KS tumors in mice (Lunardi-Iskandar, Y., et al., 1995, *Nature* 375:64-68). A

20   recent clinical study of escalating dose by intralesional injection of hCG (APL, Wyeth Ayerst) for cutaneous KS skin lesions, demonstrated tumor regression in a dose-dependent manner with 8% responding at the lowest dose (250 IU three times per week) and 83% at the highest intralesional dose (2000 IU three times per week) (Gill, P.S., et al., 1996, submitted). It is also noteworthy that

25   regression of visceral lesions occurred in 2 KS patients with advanced KS (Hermans, P., et al., 1995, *AIDS. Res. Hum. Retroviruses* S:96).

The clinical data reported herein confirms many of the beneficial effects observed in the laboratory preclinical studies. As discussed above, some preparations of hCG induced partial or complete regression of KS lesions in

175

patients treated intralesionally when the hCG (APL, Wyeth Ayerst) was used at dose levels of 250 to 2000 IU three times per week.

The intrinsic variability of native hCG preparations led to the discovery that certain β-hCG peptides (satellins) reproduce the anti viral and anti KS effects
5   *in vitro* as well as the anti-KS effect in mice with transplanted KS tumors.

6.10.7   EFFECT OF FRACTIONS OF COMMERCIAL hCG AND EARLY PREGNANCY URINE ON KAPOSI'S SARCOMA CELL GROWTH IN VITRO

10   The fractions of APL™ hCG and human early pregnancy urine were also tested for inhibition of the proliferation of cultured Kaposi's Sarcoma cells. Figure 10B depicts the results of assays of the APL™ hCG fractions for inhibition of KS Y-1 cell growth. There were three major peaks of KS cell growth inhibitory activity which eluted from the gel filtration column with apparent molecular
15   weights of approximately 40 kD, approximately 15 kD, and approximately 2-3 kD, as compared with the elutions of fractions containing hCG dimer (77 kD) and β-core protein (10 kD). A fraction containing material about the same size as the β-hCG core protein exhibited the highest level of inhibition; however, purified β-hCG core was found not to inhibit KS cell growth (data not shown).

20   Fractions of human early pregnancy urine were also assayed for inhibition of KS Y-1 cell growth. Fractions containing material which eluted from the gel filtration column with apparent molecular weights of approximately 15 kD and approximately 2-3 kD as compared with the elution of fractions containing hCG dimer (77 kD) and the β-hCG core (10 kD) as identified by immunoprecipitation
25   assay were the most effective at inhibiting KS cell growth, with the approximately 15 kD fractions having the highest activity (Figure 10E).

176

Figure 11 presents additional data on the inhibitory effects of hCG and hCG-related preparations in KS cultured cell clonogenic assays using both the KS Y-1 and KS SLK assays. Fraction 65 (from the peak eluting with an apparent molecular weight of approximately 15 kD) and 76 (from the peak eluting with an apparent molecular weight of approximately 2-3 kD) from the fractionation of both the APL™ hCG preparation (fraction 65 and 76 are represented by bars 12 and 13, respectively,) and the early pregnancy urine (fraction 65 and 76 are represented by bars 10 and 11, respectively) inhibited growth of both cell lines. The fractions containing material eluting with an apparent molecular weight of approximately 2-3 kD (i.e. fraction 76 of both fractionations) inhibited KS cell growth marginally more effectively than the fractions containing material eluting with an apparent molecular weight of approximately 15 kD (i.e. fraction 65 of both fractionations). Although the active fractions elute close to the fractions containing the β-hCG core protein, purified β-hCG core protein (bar 5) exhibited almost no inhibition of KS cell growth.

The results confirm that the APL™ hCG commercial preparation (bar 1) inhibited KS cell growth better than the other commercial hCG preparations (bars 2-4). Additionally, while native β-hCG (bar 6) inhibited KS cell growth moderately well, α-hCG, the highly purified hCG preparation CR 127 and recombinant hCG (Sigma) (bars 7-9, respectively) inhibit the KS cell growth negligibly. The results also confirm that the cyclized β-hCG peptide of amino acids 44-57 (cysteine substituted at position 44; SEQ ID NO: 26) also inhibited KS cell growth.

6.10.8 EFFECT OF COMMERCIAL hCG AND EARLY PREGNANCY URINE FRACTIONS ON KAPOSI'S SARCOMA IN VIVO

Certain fractions of the APL™ hCG and early pregnancy urine were assayed for their ability to elicit apoptosis in Kaposi's Sarcoma lesions induced by injection

177

of KS Y-1 cells in mice (n = 3 mice for each treatment). The mice were administered 100 µl subcutaneously of the particular fraction each day for one week. Table 4 presents data on the size of the Kaposi's Sarcoma lesions and the percentage of apoptotic cells within the lesion after one week of treatment with

5 fractions 60, 64, 64, 74, 82 and 85 of the APL™ hCG fractions and the unfractionated APL™ hCG preparation. The negative control treated with no hCG or fractionated hCG material exhibited little cell apoptosis or Kaposi's Sarcoma lesion regression (Table 4). Treatment with fractions 82 and 85 (containing material with apparent molecular weights smaller than

10 approximately 2-3 kD) of the APL™ hCG material also elicited almost no Kaposi's Sarcoma lesion regression or apoptosis (Table 4). The unfractionated APL™ hCG, as well as fractions 60 and 74 (fractions within the peaks containing material with apparent molecular weight of approximately 15 kD and 2-3 kD, respectively) of the APL™ hCG fractionated material, caused about 50%

15 apoptosis within the lesion and significant lesion regression (Table 4). Moreover, fractions 64 and 65 (within the peak containing material with apparent molecular weight of approximately 15 kD) of the APL™ hCG showed even higher percentage of apoptosis and more significant lesion regression than the unfractionated APL™ (Table 4).

20 Additionally, Figure 13 presents results on the effects of certain fractions of the APL™ hCG and the early pregnancy urine on KS tumors induced in mice. Those fractions from the anti-HIV and anti-KS (in vitro) peaks containing material having apparent molecular weight of approximately 15 kD (fraction 65 of the early pregnancy urine ("HAF-UF#") and fractions 62 and 65 of the APL™ hCG

25 preparation ("HAF-CF#")) and of approximately 2-3 kD (fraction 76 of the early pregnancy urine and fractions 74 and 76 of the APL™ hCG preparation) diminished KS tumors in mice as well or better than the unfractionated APL™ hCG ("APL"). However, the fractions tested that were outside these peaks of anti-HIV and anti-KS (in vitro) activities, i.e., fraction 35 of the APL™ hCG

30 (having an apparent molecular weight much larger than the hCG dimer (77 kD))

178

anti-HIV and anti-KS activity in vitro also caused regression of KS tumors induced in mice. Furthermore, phenol, an additive in the APL™ hCG preparation, had no anti-HIV activity.

6.11 EFFECTS OF hCG PREPARATIONS, hCG FRACTIONS AND β-hCG PEPTIDES ON PROSTATE, LUNG, BREAST AND KIDNEY CANCER CELLS

The hCG preparations, fractions of the early pregnancy urine and hCG APL™ SUPERDEX™ 200 fractionations (described herein), and certain β-hCG peptides were tested on prostate, lung, breast, and kidney cancer cells in vitro, and on prostate tumors in nude mice. The preparations, fractions and peptides were assayed in trypan blue dye assays by seeding cells in liquid culture and then testing for the viability of cells after treatment by trypan blue dye exclusion (viable cells do not stain for trypan blue). The preparations, fractions and peptides were also assayed using a clonogenic assay in which cells were seeded in methylcellulose in the presence or absence of test substance and then colonies were counted after a certain period of time. Cells were also examined for apoptosis by confocal microscopy.

6.11.1 EFFECT OF hCG PREPARATIONS, hCG FRACTIONS AND β-hCG PEPTIDES ON PROSTATE CANCER CELLS

The effects of hCG preparations, hCG fractions and β-hCG peptides were tested in prostate cancer cells both in vitro and in vivo. Cells were incubated in either 10% fetal bovine serum (FBS) or 3% fetal bovine serum plus hCG preparations, hCG fractions and β-hCG peptides. Table 5 presents data on the percentage of cell death as determined by the trypan blue assay.

Table 5

Treatment	10% FBS	3% FBS
PBS	7%	10%
200 IU hCG APL	38%	48%
500 IU hCG APL	44%	68%
Circ. β-hCG 44[Cys]-57 (200 μg/ml)	29%	39%
Circ. β-hCG 44[Cys]-57 (300 μg/ml)	38%	50%
Urine Fraction 60	43%	62%
Urine Fraction 64	30%	58%
Urine Fraction 74	33%	55%
Urine Fraction 23	9%	13%
Urine Fraction 80	8%	15%
APL Fraction 64	26%	42%
APL Fraction 65	27%	43%
APL Fraction 67	23%	39%
APL Fraction 72	22%	32%
APL Fraction 74	35%	52%
APL Fraction 75	28%	40%
PBS	5%	9%

Table 5 shows that the hCG APL™ preparations, fractions 60, 64 and 74 of the human early pregnancy urine SUPERDEX™ 200 fractionation and fractions 64, 65, 67, 72, 74, and 75 of the hCG APL™ fractionation and the circularized β-hCG peptide 44-57 (with cysteine substituted for position 44; SEQ ID NO:26),

all significantly caused cell death of the prostate cancer cells (all hCG fractions were added at a concentration of 200 µl/ml). Note that fractions 26 and 80 of the early pregnancy urine fractionation, which fractions did not have anti-HIV, anti-KS or pro-hematopoietic activity, did not increase prostate cancer cell

5   death.

Figures 18A-H show confocal micrographs of prostate cancer cells treated with an hCG or hCG related preparation and then stained with FITC-labelled actin monoclonal antibody to visualize the cytoskeleton and propidium iodine to visualize the cell nucleus. Condensation of the nucleus and damage to the

10   cytoskeleton are indications of apoptosis. These micrographs demonstrate increased apoptosis in cells treated with hCG APL™ (Figure 18B), the β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) (Figure 18C); and fraction 64 of the human early pregnancy urine SUPERDEX™ 200 fractionation (described herein) (Figure 18D) compared to controls (Figures 18A

15   and E-H).

Figure 19 presents data on the inhibition of colony formation in the clonogenic assay. All of the hCG APL™ fractions, as well as 200 IU and 500 IU of the hCG APL™ preparation inhibited prostate cancer cell colony formation as compared to PBS alone.

20   The circularized β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) (200 µg per day), and the hCG APL™ preparation (100 IU per day) were administered systemically to nude mice in which prostate cancers were induced and also caused apoptosis of the prostate cancer cells *in vivo* (Figures 20A-C).

6.11.2 EFFECT OF hCG PREPARATIONS, hCG FRACTIONS AND β-hCG PEPTIDES ON LUNG CANCER CELLS

The effects of hCG preparations, hCG fractions and β-hCG peptides were tested in lung cancer cells *in vitro*. Lung cancer cells were incubated with the hCG

5 preparations, hCG fractions and β-hCG peptides in both the trypan blue viability assay and the clonogenic assay. Table 6 presents data on the percentage of cell death as determined by the trypan blue assay.

Table 6

Treatment	% dead cells
PBS	11%
200 IU hCG APL	44%
500 IU hCG APL	66%
200 µg/ml β-hCG 44[Cys]-57	42%
300 µg/ml β-hCG 44[Cys]-57	59%
Urine Fraction 60	53%
Urine Fraction 64	59%
Urine Fraction 74	48%
Urine Fraction 23	13%
Urine Fraction 80	16%
APL Fraction 64	39%
APL Fraction 65	36%
APL Fraction 67	29%
APL Fraction 72	28%
APL Fraction 74	40%

| APL Fraction 75 | 38% |

Table 6 shows that the hCG APL™ preparations, fractions 60, 64 and 74 of the human early pregnancy urine SUPERDEX™ 200 fractionation and fractions 64, 65, 67, 72, 74, and 75 of the hCG APL™ fractionation and the circularized β-

5 hCG peptide 44-57 (with cysteine substituted for position 44; SEQ ID NO:26), all significantly caused cell death of the lung cancer cells. All hCG fractions were added at a concentration of 200 µl/ml. Note that fractions 26 and 80 of the early pregnancy urine fractionation, which fractions did not have anti-HIV, anti-KS or pro-hematopoietic activity (as shown herein), did not increase lung

10 cancer cell death. These results are also presented as a bar graph in Figure 21.

Figures 23A-F show confocal micrographs of lung cancer cells treated with the hCG or hCG related preparation and then stained with FITC-labelled actin monoclonal antibody to visualize the cytoskeleton and propidium iodine to

15 visualize the cell nucleus. Condensation of the nucleus and damage to the cytoskeleton are indications of apoptosis. The micrographs show increased apoptosis in cells treated with hCG APL™ (Figure 23B) and the β-hCG peptide 44-57 (with cysteine substituted at position 44; SEQ ID NO:26) (Figure 23C) compared to controls (Figures 23A and D-E).

20 Figures 22A-C present data on the inhibition of colony formation in the clonogenic assay. The hCG APL™, fractions 60, 64 and 74 of the human early pregnancy urine SUPERDEX 200 fractionation and fractions 60, 64, and 74 of the hCG APL™ fractionation all significantly inhibited lung cancer cell colony formation as compared to PBS alone controls (Figures 22A and C). Furthermore,

25 the β-hCG peptides circularized β-hCG 44-57 (with cysteine substituted for position 44; SEQ ID NO:26--"SATA2"), β-hCG 45-57 (SEQ ID NO:6--"SATA1"), and fused β-hCG peptides of 45-57::109-119 (SEQ ID NO:30--"SATAB") also

184

inhibited colony formation of the lung cancer cells as compared to controls (Figures 22A-C).

6.11.3 EFFECT OF hCG PREPARATIONS, hCG FRACTIONS AND β-hCG PEPTIDES ON BREAST CANCER CELLS

5   The effects of hCG preparations, hCG fractions and β-hCG peptides were also tested in breast cancer cells *in vitro*. Breast cancer cells were incubated with the hCG preparations, hCG fractions and β-hCG peptides in both the trypan blue viability assay and the clonogenic assay. Table 7 presents data on the percentage of cell death as determined by the trypan blue assay.

10

Table 7

Treatment	% dead cells
PBS	4%
100 IU hCG APL	34%
APL Fraction 26	3%
APL Fraction 55	13.3%
APL Fraction 65	44%
APL Fraction 76	40%
APL Fraction 82	5.4%
Urine Fraction 26	7%
Urine Fraction 55	12%
Urine Fraction 64	25%
Urine Fraction 65	42%
Urine Fraction 76	44%

Urine Fraction 82	7%
100 ug/ml Circ β-hCG 44 [Cys]-57	42%
Scramble 45-57::109-119	8.6%

Table 7 shows that the hCG APL™ preparations, fractions 60, 64 and 76 of the human early pregnancy urine SUPERDEX™ 200 fractionation and fractions 65 and 76 of the hCG APL™ fractionation and the circularized β-hCG peptide 44-57 (with cysteine substituted for position 44; SEQ ID NO:26), all significantly caused cell death of the breast cancer cells. All hCG fractions were added at a concentration of 200 µl/ml. The scrambled fused β-hCG peptide of amino acids 45-57 and 109-119 did not cause cell death. Note that fractions 26, 55 and 82 of the early pregnancy urine fractionation and fractions 55 and 82 of the hCG APL™ fractionation, which fractions did not have anti-HIV, anti-KS or pro-hematopoietic activity, did not increase breast cancer cell death.

The hCG preparation, hCG fractions and β-hCG peptides were also tested for the inhibition of breast cancer cell colony formation in the clonogenic assay. The data is presented in Table 8.

Table 8

Treatment	% inhibition
PBS	98.3%
hCG-APL-100 IU/ml	50%
APL Fraction 26	0%
APL Fraction 55	12.5%
APL Fraction 65	50.4%

186

APL Fraction 76	56.3%
Urine Fraction 26	0%
Urine Fraction 55	15.2%
Urine Fraction 65	51%
100 ug/ml Circ β-hCG 44[Cys]-57	45%
Scramble 45-57::109-119	0%

The hCG APL™, fraction 65 of the human early pregnancy urine SUPERDEX™ 200 fractionation and fractions 65 and 76 of the hCG APL™ fractionation, and the circularized β-hCG peptide 44-57 (with cysteine substituted for position 44; SEQ ID NO:26) all significantly inhibited colony formation of the breast cancer cells as compared to PBS alone controls. The scrambled fused peptide of amino acids 45-57 (SEQ ID NO:6) and 109-119 (SEQ ID NO:7) did not significantly inhibit of colony formation. Note that fractions 26 and 55 of the early pregnancy urine fractionation and fractions 26 and 55 of the hCG APL™ fractionation, which fractions did not have anti-HIV, anti-KS or pro-hematopoietic activity, did not inhibit breast cancer colony formation.

6.11.4 EFFECT OF hCG PREPARATIONS ON KIDNEY CANCER CELLS

Two different concentrations of the hCG APL™ preparation were tested for the ability to induce apoptosis in kidney cancer cells. Figures 24A-I show confocal micrographs of kidney cancer cells treated with the hCG APL™ preparation and then stained with FITC-labelled actin monoclonal antibody to visualize the cytoskeleton and propidium iodine to visualize the cell nucleus. Condensation of the nucleus and damage to the cytoskeleton are indications of apoptosis. The micrographs show increased apoptosis in cells treated with 100 IU hCG APL™ (Figures 24D-F) and even higher levels of apoptosis in cultured kidney cancer

187

cells treated with 300 IU hCG APL™ (Figures 24G-I) compared to controls treated only with PBS (Figures 24A-C).

6.12 EFFECT OF FRACTIONS OF A COMMERCIAL hCG PREPARATION AND EARLY PREGNANCY URINE ON HIV GENE EXPRESSION IN HIV-I TRANSGENIC MICE

Finally, fractions from the APL™ hCG and early pregnancy urine fractionation were tested for their ability to improve survival and to reduce HIV-I gene expression in HIV-I transgenic mice. Figure 1E presents results of administration of 200 µl of fraction 61 of the APL™ hCG fractionation (bar 4) and fraction 65 of the early pregnancy urine fractionation (bar 5). These two fractions, both of which are within the peak of anti-HIV and anti-KS activity that contains material with an apparent molecular weight of approximately 15 kD, significantly suppressed HIV-I gene expression in the HIV-I transgenic mice (as measured in the skin and the kidney) in comparison to PBS alone (bar 1), β-hCG core peptide (bar 2) and α-hCG subunit (bar 3).

HIV-I transgenic pups administered hCG and hCG-related preparations for the first 5 days after birth through the mothers milk were monitored for survival at 10 days after birth. All the pups receiving the unfractionated APL™ hCG (bar 2), fraction 65 from both the APL™ hCG fractionation and the early pregnancy urine fractionation (bars 4 and 5, respectively), and fraction 76 of the early pregnancy urine fractionation (bar 6) survived (figure 14). These fractions 65 and 76 are within the anti-HIV and anti-KS activity peaks containing material with apparent molecular weights of approximately 15 kD and 2-3 kD, respectively. None of the pups receiving fraction 26 of either the APL™ hCG or the early pregnancy urine (bars labelled as "HAF-CF#26" and "HAF-UF#26", respectively) survived (figure 14). These fractions contain material with an apparent molecular weight larger than the hCG heterodimer (77 kD). Figure 14 also presents data showing that native β-hCG (bar 1) and the β-hCG peptide of

188

amino acids 44-57 (with cysteine substituted at position 44; SEQ ID NO: 26--bar 7) and to a lesser extent LH (bar 3) promote survival of the HIV-I transgenic pups while the α-hCG subunit, the highly purified hCG preparation CR127, recombinant β-hCG and the β-hCG core protein (bars labelled as "ahCG",

5   "CR127", "rβhCG", and "b-core", respectively) failed to promote survival of the transgenic mouse pups.

The above-described experiments demonstrate that the factor(s) responsible for the anti-HIV and anti-KS activities can be further isolated from the hCG preparations by gel filtration on a SUPERDEX™ 200 gel filtration column. The

10   factor(s) were fractionated from both the commercial APL™ hCG preparation and urine from women in early pregnancy (first trimester). The fractions of highest anti-HIV and anti-KS activity contained material eluting from the gel filtration column with an apparent molecular weights of approximately 40 kD, 15 kD and 2-3 kD. Although certain active fractions contained material of approximately

15   the size of the β-hCG core protein (~10 kD), purified β-hCG core protein was found to have neither anti-HIV nor anti-KS activity. The fractions exhibiting anti-HIV and anti-KS activity in vitro also had pro-hematopoietic activity in vitro, caused regression of KS tumors induced in mice, and promoted survival and reduced HIV-I gene expression in HIV-I transgenic mice. Furthermore,

20   phenol, an additive in the APL™ hCG preparation, had no anti-HIV activity.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are

25   intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

SEQUENCE LISTING

```
        (1)  GENERAL INFORMATION
        (i)  APPLICANT: Gallo, Robert C.
                        Bryant, Joseph
                        Lunardi-Iskandar, Yanto
        (ii) TITLE OF THE INVENTION: TREATMENT AND PREVENTION OF
                HIV INFECTION BY ADMINISTRATION OF
                DERIVATIVES OF HUMAN CHORIONIC GONADOTROPIN
        (iii) NUMBER OF SEQUENCES: 37
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          (D) STATE: NY
          (E) COUNTRY: USA
          (F) ZIP: 10036/2711
        (v)  COMPUTER READABLE FORM:
          (A) MEDIUM TYPE: Diskette
          (B) COMPUTER: IBM Compatible
          (C) OPERATING SYSTEM: DOS
          (D) SOFTWARE: FastSEQ Version 2.0
        (vi) CURRENT APPLICATION DATA:
          (A) APPLICATION NUMBER:
          (B) FILING DATE: 24-JUN-1997
          (C) CLASSIFICATION:
        (vii) PRIOR APPLICATION DATA:
          (A) APPLICATION NUMBER: USSN 08/709,948
          (B) FILING DATE: 09-SEP-1996
          (A) APPLICATION NUMBER: USSN 08/669,681
          (B) FILING DATE: 24-JUN-1996
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          (C) TELEX: 66141 PENNIE
    (2) INFORMATION FOR SEQ ID NO:1:
        (i)  SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 539 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: DNA
        (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 26..520
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
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   52
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                                -20                        -15


TTG CTG CTG CTG AGC ATG GGC GGG ACA TGG GCA TCC AAG GAG CCG CTT
   100
Leu Leu Leu Leu Ser Met Gly Gly Thr Trp Ala Ser Lys Glu Pro Leu
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190

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        -10                      -5                        1
CGG CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG
148
Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu
            10                      15                      20
GGC TGC CCC GTG TGC ATC ACC GTC AAC ACC ACC ATC TGT GCC GGC TAC
196
Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr
                    25              30                      35
TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT
244
Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
            40                      45                      50
CAG GTG GTG TGC AAC TAC CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC
292
Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe Glu Ser Ile Arg Leu
        55                      60              65
CCT GGC TGC CCG CGC GGC CTG AAC CCC GTG GTC TCC TAC GCC GTG GCT
340
Pro Gly Cys Pro Arg Gly Leu Asn Pro Val Val Ser Tyr Ala Val Ala
70                      75                      80              85
CTC AGC TGT CAA TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG
388
Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly
                    90                      95                  100
GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC
436
Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp
                105                     110                     115
TCC TCT TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA
484
Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg
            120                     125                     130
CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAAAGGCTTC
530
Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
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TCAATCCGC
539
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(2) INFORMATION FOR SEQ ID NO:2:
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           (D) TOPOLOGY: linear
      (ii) MOLECULE TYPE: protein
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Gly Thr Trp Ala Ser Lys Glu Pro Leu Arg Pro Arg Cys Arg Pro Ile
                1                       5                       10
Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr
            15                      20                      25
Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val
        30                      35                      40
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg
45                      50                      55                      60
Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Leu
                65                      70                      75
Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu
            80                      85                      90
Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu
        95                      100                     105
Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro
```

191

```
          110                    115                    120
                                                       Gly Pro Ser Asp Thr
Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro                          140
125                    130                    135
Pro Ile Leu Pro Gln
                    145
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(2) INFORMATION FOR SEQ ID NO:3:
    (i) SEQUENCE CHARACTERISTICS:
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        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1                   5                   10
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(2) INFORMATION FOR SEQ ID NO:4:
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        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
1                   5                   10
```

(2) INFORMATION FOR SEQ ID NO:5:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Gly Val Leu Pro Ala Leu Pro
1                   5
```

(2) INFORMATION FOR SEQ ID NO:6:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 13 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1                   5                   10
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(2) INFORMATION FOR SEQ ID NO:7:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 11 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: circular, linear
    (ii) MOLECULE TYPE: peptide
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
1                   5                   10
```

(2) INFORMATION FOR SEQ ID NO:8:
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        (A) LENGTH: 13 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro
1                   5                   10
```

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        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

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            (ii) MOLECULE TYPE: peptide
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            1               5                   10
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                (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: peptide
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
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            (ii) MOLECULE TYPE: peptide
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(2) INFORMATION FOR SEQ ID NO:12:
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            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
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(2) INFORMATION FOR SEQ ID NO:13:
            (i) SEQUENCE CHARACTERISTICS:
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                (B) TYPE: amino acid
                (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: peptide
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
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            1               5
(2) INFORMATION FOR SEQ ID NO:14:
            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 8 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: peptide
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
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            1               5
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                (B) TYPE: amino acid
                (D) TOPOLOGY: linear
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            Leu Gln Gly Val Leu Pro Ala Leu Pro Gln
            1               5                   10
(2) INFORMATION FOR SEQ ID NO:16:
            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 11 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE: peptide
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193

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1   5   10
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        (D) TOPOLOGY: linear
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1   5   10
(2) INFORMATION FOR SEQ ID NO:18:
    (i) SEQUENCE CHARACTERISTICS:
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        (D) TOPOLOGY: linear
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1   5   10
(2) INFORMATION FOR SEQ ID NO:19:
    (i) SEQUENCE CHARACTERISTICS:
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        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
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    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
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1   5
(2) INFORMATION FOR SEQ ID NO:20:
    (i) SEQUENCE CHARACTERISTICS:
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        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
Gly Val Leu Pro Ala Leu Pro Gln Val
1   5
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        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
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Gly Val Leu Pro Ala Leu Pro Gln Val Val
1   5   10
(2) INFORMATION FOR SEQ ID NO:22:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 12 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
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Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn
1   5   10
(2) INFORMATION FOR SEQ ID NO:23:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 98 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: peptide
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

194

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Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg
1               5                   10                  15

Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Leu Asn Pro Val
            20                  25                  30

Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg
        35                  40                  45

Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp
    50                  55                  60

Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser
65                  70                  75                  80

Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu
            85                  90                  95

Pro Gln
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(2) INFORMATION FOR SEQ ID NO:24:
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      (B) TYPE: amino acid
      (D) TOPOLOGY: linear
  (ii) MOLECULE TYPE: peptide
  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

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1               5                   10                  15

Arg Gly Leu Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln
            20                  25                  30

Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp
        35                  40                  45

His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser Ser
    50                  55                  60

Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro
65                  70                  75                  80

Ser Asp Thr Pro Ile Leu Pro Gln
            85
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      (B) TYPE: amino acid
      (D) TOPOLOGY: linear
  (ii) MOLECULE TYPE: peptide
  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

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1               5                   10                  15

Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr
            20                  25                  30

Pro Ile Leu Pro Gln
        35
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      (B) TYPE: amino acid
      (D) TOPOLOGY: circular

195

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           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
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     1               5                   10
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           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
     Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
     1               5                   10
             (2) INFORMATION FOR SEQ ID NO:29:
         (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 12 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
     Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser
     1               5                   10
             (2) INFORMATION FOR SEQ ID NO:30:
         (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 24 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
     Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Thr Cys Asp
     1               5                   10                  15
     Asp Pro Arg Phe Gln Asp Ser Ser
             20
             (2) INFORMATION FOR SEQ ID NO:31:
         (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 23 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
     Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Leu Gln Gly Val Leu Pro
     1               5                   10                  15
     Ala Leu Pro Gln Val Val Cys
             20
             (2) INFORMATION FOR SEQ ID NO:32:
         (i) SEQUENCE CHARACTERISTICS:
           (A) LENGTH: 23 amino acids
           (B) TYPE: amino acid
           (C) STRANDEDNESS: single
           (D) TOPOLOGY: linear
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196

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        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Leu Thr Cys Asp Asp
1               5                   10                  15
Pro Arg Phe Gln Asp Ser Ser
            20
        (2) INFORMATION FOR SEQ ID NO:33:
        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 34 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly
1               5                   10                  15
Cys Pro Val Cys Ile Thr Val Asn Thr Thr Ile Cys Ala Gly Tyr Cys
            20                  25                  30
Pro Thr

        (2) INFORMATION FOR SEQ ID NO:34:
        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp
1               5                   10                  15
Val Arg Phe Glu
            20
        (2) INFORMATION FOR SEQ ID NO:35:
        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 9 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
Val Leu Pro Ala Leu Pro Gln Val Val
1               5
        (2) INFORMATION FOR SEQ ID NO:36:
        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 14 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:
Cys Val Ala Gln Pro Gly Pro Gln Val Leu Leu Val Leu Cys
1               5                   10
        (2) INFORMATION FOR SEQ ID NO:37:
        (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 15 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
        (ii) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
Cys Val Ala Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys
1               5                   10                  15
```